JOURNAL OF DAIRY SCIENCE

Contents

Thyroprotein Feeding to Dairy Cows During Successive Lactations. J. W. Thomas and L. A. Moore 657

A Comparison of Homogenized Whole Milk and Egg Yolk-Phosphate as Diluters for Bull Semen. W. H. Dreher and J. H. Webb 673

Production Characteristics of Crossbred, Backcross, and Purebred Red Sindhi Cattle in the Gangetic Plains Region. H. H. Stonaker, O. P. Agarwala, and D. Sundaresan 678

Estimates of Genetic Changes in an Indian Herd of Red Sindhi Dairy Cattle. H. H. Stonaker 688

The Extracellular Lipases of Some Gram-Negative Non-Sporeforming Rod-Shaped Bacteria. S. A. Nashif and F. E. Nelson 698

Contamination of Milking Machine Teat-Cup Liners as Affected by Inconspicuous Deterioration of the Interior Surfaces. T. J. Claydon 707

A High Roughage System for Raising Calves Based on the Early Development of Rumen Function. I. Effect of Variations in the Ration on Growth, Feed Consumption, and Utilization. J. W. Hibbs, W. D. Pounden, and H. R. Conrad 717

The Effects of Freezing Bovine Spermatozoa in Extenders Containing Antibacterial Agents. H. O. Dunn, G. L. Larson, and E. L. Willett 728

Lipid and Lipoprotein Constituents of Egg Yolk in the Resistance and Storage of Bull Spermatozoa. R. F. Kampschmidt, D. T. Mayer, and H. A. Herman 733

Antibiotics in Rumen Digestion and Synthesis. III. The Effect of Aureomycin on Rumen Microorganisms, with Special Reference to the Streptococci and Coliform Groups. C. M. Chance, C. K. Smith, C. F. Huffman, and C. W. Duncan 743

Baking Properties of Frozen Condensed Skimmilk. L. V. Rogers, R. W. Bell, and T. J. Mucha 752

Influence of Rate and Temperature of Cooking on the Time Required to Manufacture Cheddar Cheese from Pasteurized Milk. W. W. Overcast, E. R. Jarman, and T. W. Albrecht 757

Block vs. Loose Salt Consumption by Dairy Cattle. S. E. Smith, F. W. Lengemann, and J. T. Reid 762

Studies of Heated Milk. V. The Reaction of Lactose with Milk Protein as Shown by Lactose-1-C^{14}. Stuart Patton and Robert J. Flipse 766

The Effect of Relaxin and Mechanical Dilation on the Bovine Cervix. Edmund F. Graham and Arthur E. Dracy 772

People and Events 778

Abstracts of Literature A-71

Vol. XXXVI, No. 7, July, 1953

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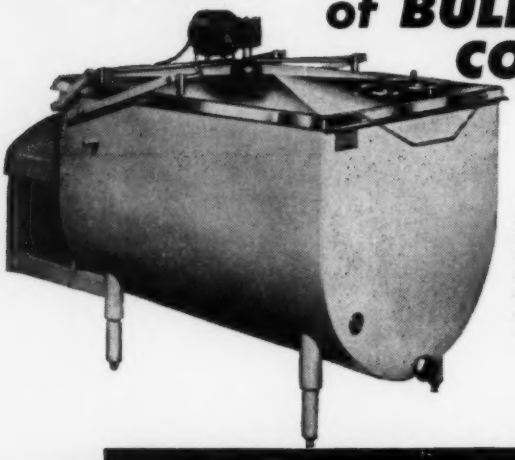

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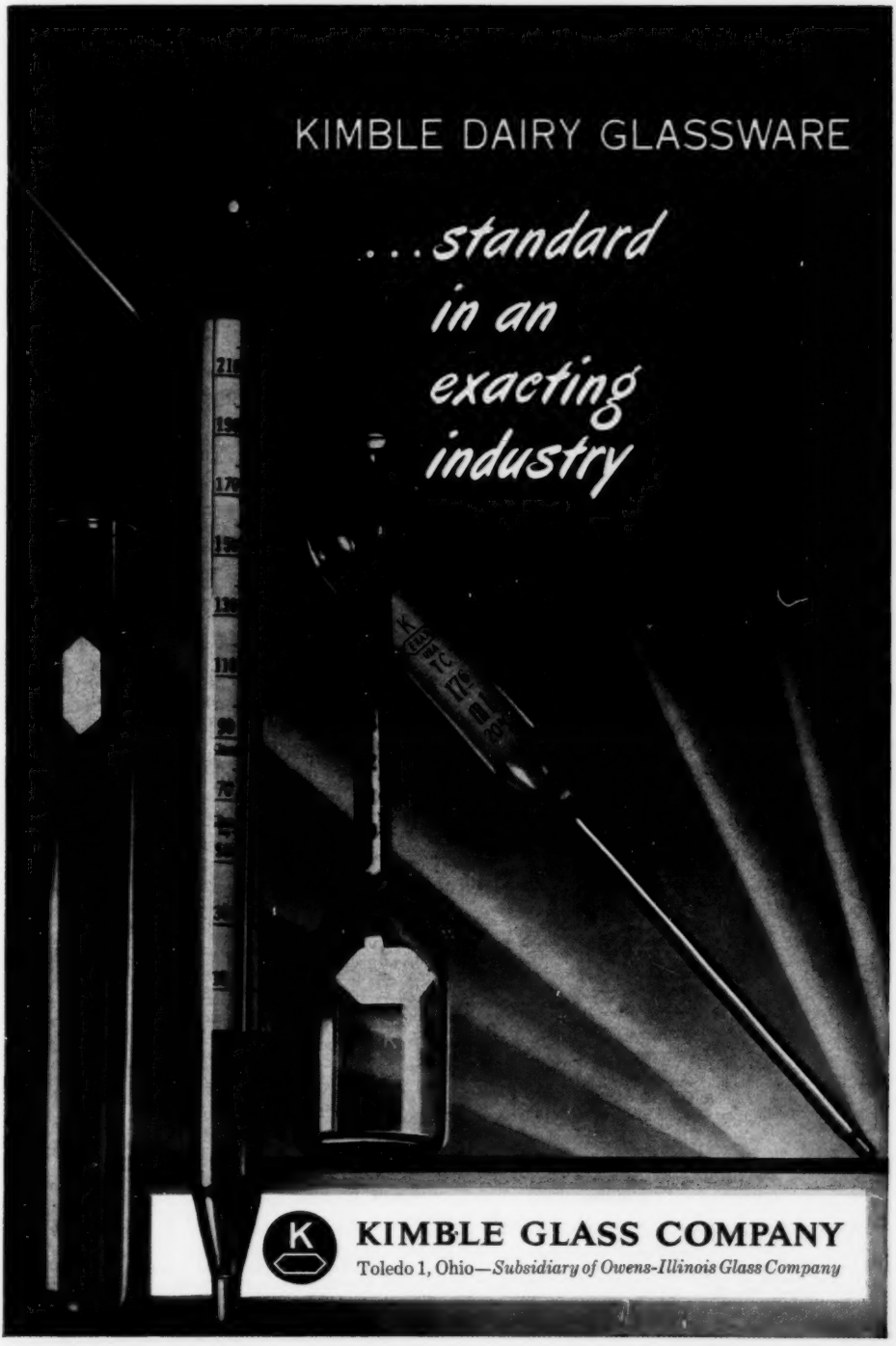

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OFFICIAL ORGAN OF
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Published at
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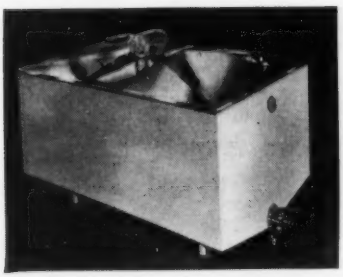

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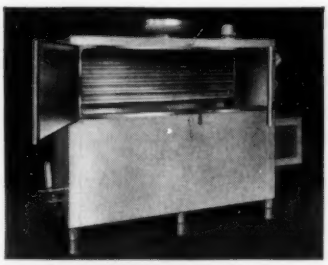

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXVI JULY, 1953 NUMBER 7

THYROPROTEIN FEEDING TO DAIRY COWS DURING SUCCESSIVE LACTATIONS

J. W. THOMAS AND L. A. MOORE

Bureau of Dairy Industry, U. S. Department of Agriculture
Washington, D. C.

A large number of reports have been published on the feeding of thyroprotein to animals since the product has become available for experimental and practical use. It had been shown previously that dried or fresh thyroid tissue when fed to the cow would cause an increase in the level of milk production (9, 11, 12). Thyroxine was shown to be the active ingredient causing the increased milk yield (10). Investigations after this time were performed mainly to demonstrate that there was an increase in the milk and fat production after the thyroid or thyroprotein material was fed. Since then many trials have been carried out over short periods of time and were concerned with many and varied aspects of the subject. Several reviews have appeared, in which both the subject material and the literature references have been adequately given (7, 17).

This experiment was set up to obtain information on cows that were fed thyroprotein for each of several successive lactations. It was originally designed to keep the cows on this treatment as long as they remained healthy and in the herd.

EXPERIMENTAL

Twenty first-calf heifers of the Holstein and Jersey breeds were started in the experimental group that received thyroprotein. During each lactation thyroprotein [1] was fed from the 50th day postpartum until 90 days before the next expected parturition. Fifteen of these cows completed two lactations, and nine completed three lactations. Four of the cows remained in the herd through five lactations, and three completed six lactations. The length of thyroprotein feeding averaged 300, 311, 252, 248, 318, and 288 days during the first through the sixth lactations, respectively. The cows were fed thyroprotein at the rate of 1.0 or 1.5 g. per hundredweight per day except two cows that were fed at the rate of 0.6 g. during their first lactation.

Eight first-calf heifers were started in the control groups. These cows received exactly the same feed and treatment as the cows in the thyroprotein group except that they were not fed thyroprotein. Six of these cows completed

Received for publication December 10, 1952.

[1] "Protamone" brand of iodinated casein obtained from Cerophyll Corp., Kansas City, Mo., through the courtesy of Dr. W. R. Graham, Jr.

two lactations, and five completed three lactations under these experimental conditions. Two of these cows remained on the experiment through five lactations, and one cow remained through six lactations.

In making comparisons of data between those of control cows and that of cows fed thyroprotein there were, at times, different numbers of animals used in some of these comparisons in the text. Some of these differences occurred when injury, ketosis, mastitis or termination of the experiment intervened during the course of a lactation period. Thus, the data used in comparing complete lactations were made up of a smaller number of cows than when data on partial lactations were compared. Similarly, the data comparing milk production were sometimes made up of a smaller number of cows than were the data comparing fat tests.

All cows were fed 7.5 lb. of U. S. No. 1 alfalfa, 7.5 lb. of timothy hay, and 15 lb. of corn silage per 1,000 lb. of body weight per day. This roughage allowance was calculated in early lactation and remained at that level of intake throughout the lactation. The remainder of the nutrient allotment for each cow was made up in the form of a concentrate mixture consisting of wheat bran, 40 parts; ground yellow corn, 40 parts; linseed meal, 20 parts; and ordinary salt, 1 part.

When thyroprotein was fed during the first lactation, nine of the cows in the thyroprotein group were fed a total digestible nutrient (TDN) intake equal to Morrison's maximum requirement (15). Eleven were fed TDN at 125 per cent of their requirement when they were receiving thyroprotein. During all later lactations all the cows were fed TDN at 125 per cent of requirement whenever thyroprotein was fed.

During the first lactation four cows in the control group were fed TDN at requirement and four at 125 per cent of requirement. During the second lactation there were three control cows on each level of feeding. In all later lactations the control cows were fed TDN at 125 per cent of requirement from the 50th to the 310th day of lactation, but actual consumption was frequently only 105 to 110 per cent of requirement.

Adjustments in the amount of grain were made at 10-day intervals and were based on the cows' body weights and milk production over similar periods of time. In late lactation or when the cows were dry, the grain allowance was not decreased below a minimum of 0.5 lb. per hundredweight. Daily feed records of amounts fed and refused were used for calculating TDN consumption of each cow based on appropriate values for the feeds fed (15). Composite milk samples were obtained for butterfat tests by the Babcock method on the 5th and 6th days of each 10-day period.

Electrocardiograph tracings were taken periodically, and the results of this study have been reported elsewhere (21).

RESULTS

Effect on milk production. In 58 lactations of this experiment the average fat corrected milk (FCM) for the month following the beginning of feeding of thyroprotein was 108 per cent of the average for the preceding month. In 24

lactations of control cows the corresponding figure was 92 per cent. The average daily increase for the 58 lactations when thyroprotein was fed was 2.9 lb. FCM and the average decrease for the 24 lactations of the control cows was 3.4 lb. This was a net difference for the first post-experimental month of thyroprotein feeding of 6.3 lb., or about 16 per cent, between control and thyroprotein-fed cows. In seven of the 58 lactations there was a decrease in average production for the month after initiating thyroprotein feeding. The effect noted on milk production for the month after feeding thyroprotein was similar to other previous observations (1, 3, 4, 5, 7, 8, 14, 16, 17, 18, 19, 22).

The increase in the first month of thyroprotein feeding remained fairly constant from the first to the third lactations, inclusive. The average percentage increase was practically the same (10 per cent) for each lactation. The average increase expressed in pounds of FCM per day was more for the cows in their second and third lactations than it was for the same cows in their first lactations. In the sixth lactation four cows had a smaller increase in production than in any other.

During the first lactation the actual production of eight control cows was 8,683 lb. of FCM and that of 19 cows fed thyroprotein was 8,026 lb. The cows in the control group had the lowest average nutrient intake during the lactation, 105 per cent compared to 111 per cent. The average production, on a mature equivalent basis, for six control cows was 10,447 and 10,549 lb. in their first and second lactations, respectively. The average for 15 cows fed thyroprotein was 10,129 and 9,534 lb. of FCM for the two respective lactations. The decrease in production of 595 lb. from the first to the second lactation of the cows fed thyroprotein was not statistically significant. However it occurred concomitantly

TABLE 1

Average 305-day FCM production on a mature equivalent basis by control cows and thyroprotein-fed cows and TDN intake expressed as a % of requirement for the period

	Control	Thyro-protein	Control	Thyro-protein	Control	Thyro-protein	Control	Thyro-protein
No. of cows —	8	19	6	15	5	6	2	3
1st lactation								
Milk *(lb.)*	11,144	10,122	10,447	10,129	9,657	9,272	9,915	10,338
TDN *(%)*	105	111	105	110	103	103	98	111
2nd lactation								
Milk *(lb.)*	.....	.....	10,549	9,534	10,090	9,012	9,792	10,108
TDN *(%)*	.....	.....	108	119	106	117	103	119
3rd lactation								
Milk *(lb.)*	.....	.....	.....	.....	11,491	8,355	12,196	9,468
TDN *(%)*	.....	.....	.....	.....	107	114	105	119
4th lactation								
Milk *(lb.)*	.....	.....	.....	.....	.....	.....	11,348	9,048
TDN *(%)*	.....	.....	.....	.....	.....	.....	100	113
5th lactation								
Milk *(lb.)*	.....	.....	.....	.....	.....	.....	11,150	10,158
TDN *(%)*	.....	.....	.....	.....	.....	.....	106	114
6th lactation								
Milk *(lb.)*	.....	.....	.....	.....	.....	.....	.....	10,436
TDN *(%)*	.....	.....	.....	.....	.....	.....	.....	111

with an increase in rate of TDN consumption, which should have the effect of increasing the level of production.

The data in Table 1 show that the control cows maintained or increased their level of production over successive lactations. In each lactation the control cows had approximately the same average rate of TDN intake. The cows fed thyroprotein showed a definite trend toward a decrease in their level of production during the later successive lactations, with an average increase in rate of TDN consumption. This was not true for every individual cow, but the majority showed this tendency. The evidence indicates that the use of thyroprotein over successive lactations is contraindicated.

In this experiment the cows did not receive thyroprotein during the first 50 days of the lactation. The amount of FCM produced and the fat test during the first 50 days of the first lactation are given in Table 2. The values for the following lactations have been expressed as a percentage of the first lactation for both the thyroprotein and the control group of cows. The control group of cows showed a greater increase in the second lactation as compared to the first for both milk production and fat test during this period than was shown by the cows fed thyroprotein. The difference between the changes in butterfat test of these two groups between the first and second lactations was highly significant ($P < 0.01$). The FCM production difference was significant at only $P = 0.07$. The difference in FCM between the first and third lactations of the control and

TABLE 2

Average FCM production and fat test for cows during the first 50-day period of successive lactations

Group	No. of cows	Lactation 1st	2nd	3rd	4th	5th	6th
		Production test	% of 1st	% of 1st	% of 1st	% of 1st	% of 1st
		Av. butterfat test during first 50 days of successive lactations (%)					
Control	7	4.38	106	.....	.....	.....	.....
Thyroprotein	18	4.55	92	.....	.....	.....	.....
Control	5	4.59	108	112	.....	.....	.....
Thyroprotein	9	5.02	94	96	.....	.....	.....
Control	4	4.39	105	115	115	.....	.....
Thyroprotein	8	5.11	95	97	99	.....	.....
Control	2	4.46	101	103	109	111	.....
Thyroprotein	3	4.91	90	88	96	97	104
		Av. FCM produced during first 50 days of successive lactations (lb.)					
Control	7	1701	125	.....	.....	.....	.....
Thyroprotein	18	1666	109	.....	.....	.....	.....
Control	5	1754	119	148	.....	.....	.....
Control	3	1582	120	163	155	.....	.....
Thyroprotein	7	1481	113	118	118	.....	.....
Thyroprotein	2	1354	138	142	148	175	176

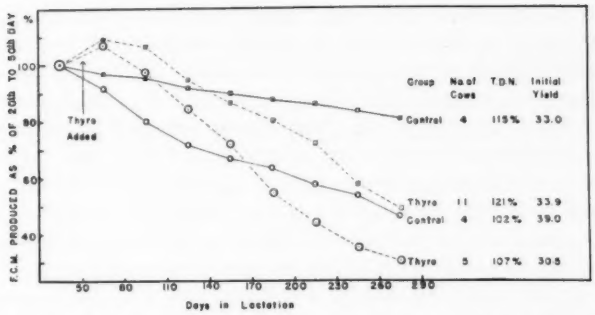

FIG. 1. The effect of feeding thyroprotein and extra TDN (singly and combined) beginning at 50 days postpartum, on the persistency of milk production during the first lactation.

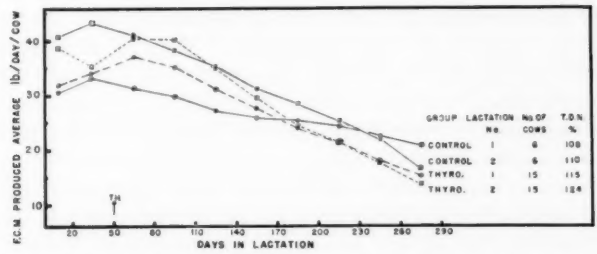

FIG. 2. Level of FCM production during the entire first and second lactations of 6 control cows and 15 cows receiving thyroprotein.

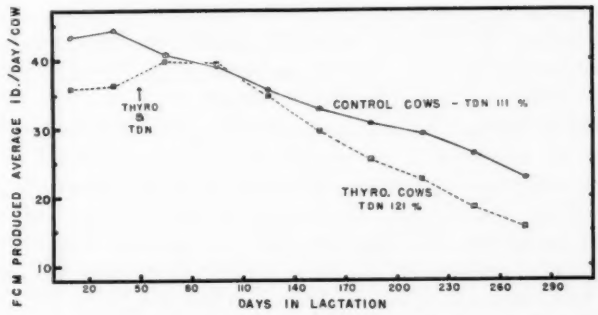

FIG. 3. Average actual FCM production by 30 day periods for all cows receiving thyroprotein and extra TDN (43 lactations) and for control cows receiving extra TDN (17 lactations).

the thyroprotein-fed cows was significant ($P = 0.02$). In the latter lactations there was a large variation among the individual cows in this comparison. A comparison between other normal cows in this herd and cows fed thyroprotein

showed a similar significant difference. This comparison indicates that there was a relative decrease in the productive capacity during the first 50 days of a lactation following one in which thyroprotein was fed, as in this experiment. Further evidence to support this finding is shown in the initial production levels in Figures 2 and 3. In Figure 2 the control cows averaged about 11 lb. more per day during the 20th to the 50th day of the second lactation (second point on graph) than they did during the first lactation, whereas the cows fed thyroprotein averaged only 2 lb. FCM more per day. Thus the control cows increased their initial production in the following lactation by a larger amount than did the cows fed thyroprotein. In the first lactation the initial production of both groups was approximately the same but when all lactations are averaged together as in Figure 3 the initial level of cows in the thyroprotein group was lower than that of the control group. It is possible that the cow's thyroid gland may still be functioning at a subnormal rate at this time. A very limited amount of data on the protein bound iodine level indicated that it was low in two cows of the thyroprotein group during this early stage of lactation.

Early in this experiment it was reported (22) that the effect of thyroprotein on milk production, body weight, and heart rate over the entire lactation was dependent on the level of nutrients fed. The effect of feeding thyroprotein and extra nutrients (combined and alone) on the FCM production throughout the first lactation is shown in Figure 1.

A much greater persistency was observed when the extra nutrients were added to the ration after the 50th day as compared to the cows not fed the extra nutrients. This occurred both in the normally-fed cows and in the thyroprotein-fed cows. The initial level of production during the 20th to the 50th day and the average rate of TDN consumption expressed as a percentage of requirement from the 50th to the 305th day of lactation also are given in Figure 1. The increase in milk production of cows fed thyroprotein apparently disappeared after mid-lactation. The data in Figure 1 indicates that the feeding of extra nutrients alone generally would be as satisfactory as feeding thyroprotein and extra nutrients during the last 255 days of the first lactation.

The actual production for the corresponding periods of the first and second lactations of six control and 15 thyroprotein-fed cows is shown in Figure 2. Greater persistency during the first lactation, as compared to the second, was shown by both groups of cows. The average trends in production for cows in lactations when they all received extra TDN is shown in Figure 3. For each group there was approximately the same percentage of cows in each of the six lactations. The average production of the thyroprotein group for the 2 months after initiation of thyroprotein feeding was about 109 per cent of the preexperimental month.

The data presented in Table 1 and Figures 1, 2, and 3 indicate that the feeding of thyroprotein over the major portion of the lactation period, for one or for successive lactations, results in no greater amounts of FCM production for the entire lactation than would be produced by a comparable group of cows similarly fed but not receiving thyroprotein. The administration of thyroprotein and

TABLE 3

Average change in butterfat test from the 20th to 50th day as compared to the 60th to 90th day in successive lactations of control cows and cows fed thyroprotein

Group	No. of cows	Lactation					
		1st	2nd	3rd	4th	5th	6th
		(%)	(%)	(%)	(%)	(%)	(%)
Control	8	+0.12	.....	.....	.....	.....	.....
Thyroprotein	20	+0.32	.....	.....	.....	.....	.....
Control	6	+0.20	—0.15	.....	.....	.....	.....
Thyroprotein	16	+0.38	+0.56	.....	.....	.....	.....
Control	5	+0.28	—0.17	—0.14	.....	.....	.....
Thyroprotein	9	+0.34	+0.52	+0.32	.....	.....	.....
Control	2	+0.07	—0.12	—0.06	—0.18	—0.39	+0.04[a]
Thyroprotein	8	+0.36	+0.50	+0.31	+0.11	.....	.....
Thyroprotein	3	+0.64	+1.15	+0.95	+0.36	+0.20	—0.07

[a] One cow only.

extra nutrients to a given cow usually, but not always, elicited an increase in the level of milk production for the following month.

Effect on the fat test of the milk. The effect on the fat test immediately after thyroprotein was fed was determined. The average fat test increase for the first month in 60 lactations when cows were fed thyroprotein was 0.33 per cent. This increase was significant ($P = 0.05$). The average fat test decrease for 24 lactations of cows in the control group was 0.07 per cent.

The values in Table 3 show the average increase for the first month during the various lactations. The increase was larger for the thyroprotein-fed cows in their second lactation than for any other lactation. The increase in butterfat test after the second lactation was progressively smaller. In the sixth lactation none of the four cows showed a noticeable increase after the thyroprotein was fed. Neither did one cow in her seventh and eighth lactations show any increase in fat test. The values for the control cows indicate that there was little change in the fat tests between these two periods over the lactations, especially after the first lactation. These data on the effect of fat test for the month after feeding thyroprotein are comparable to the findings of most other investigators (1, 2, 3, 4, 5, 7, 8, 14, 16, 17, 18, 22).

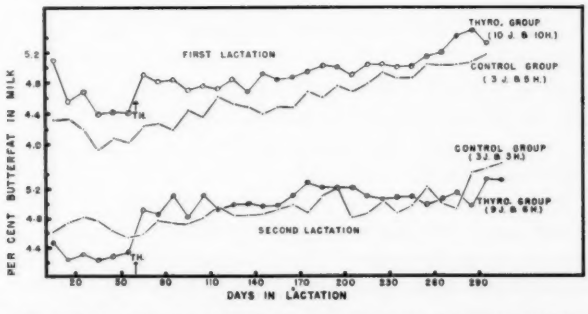

FIG. 4. The effect of thyroprotein feeding on the butterfat content of the milk.

The average butterfat tests by 10-day periods over the entire first and second lactations are presented in Figure 4. The control and thyroprotein groups are shown for both the first and second lactation. The immediate increase following the initiation of thyroprotein feeding was very evident. Figure 4 and Table 3 show that the response during the second lactation was larger than during the first lactation. The data for the third lactation (not illustrated) show that the two groups were indistinguishable except for the 60th to the 80th day, when the immediate effect of thyroprotein was evident. Normal cows (22) and control cows in this experiment always had a gradual increase in fat test as the lactation progressed. By comparing the lactational trend in fat percentage of the control cows and other normal cows to that of cows fed thyroprotein, the evidence indicates that the effect of thyroprotein on the fat test was transient. After mid-lactation the fat test was not above the level that it normally might have been if no thyroprotein had been fed.

The amount of nutrients fed had an effect on the manner in which thyroprotein influenced the fat test. There was no essential difference in the immediate effect, but the effect of level of feeding was noted during the second and third months of thyroprotein feeding. In the first lactation the immediate increase in fat test was 0.38 per cent for the nine cows receiving TDN at 100 per cent of requirement and 0.27 for the 11 cows fed extra TDN. From the 85th to the 135th day of lactation, the fat test on the nine cows fed at requirement gradually decreased by 0.15 per cent, whereas the fat test for the 11 cows fed at 125 per cent of requirement increased slightly (0.07) during this period. An examination of the data in Figure 4 shows that the average test for first lactation cows fed thyroprotein decreased from the 65th to the 135th day. This decrease probably was due to the fact that nine of the 20 cows were fed at requirement and had a decrease in fat test over this period. In all other lactations, when all cows were fed extra TDN, the fat content was constant or increased slightly during this period. However, during this period the fat test of cows fed thyroprotein and extra TDN did not increase as rapidly as did the fat test of the control cows.

The cows fed thyroprotein had their highest average tests and the control group had their lowest average tests during the first lactation. This was true for both the 50th to the 305th day segment and for the entire 305-day period of the successive lactations.

These data, which are similar to the majority of other published data for short-time experiments, indicated that there was a small increase in fat test after thyroprotein was fed. The data from this study indicate also that there was little effect on fat test when the entire lactation period was considered. The observed decrease in average fat test in the later lactations of cows fed thyroprotein would be undesirable.

Effect on body weight. Previous publications (1, 3, 4, 5, 7, 8, 14, 17, 22) have shown that cows given thyroprotein and fed TDN at requirement showed an excessive decrease in body weight. The feeding of TDN at 125 per cent of requirement resulted in near normal changes in body weight (22). The results in this experiment verify and extend these findings.

The average body weight for the three 10-day periods before thyroprotein feeding was initiated was compared with the average body weight for the three 10-day periods at 100, 110, and 120 days after the starting of thyroprotein feeding. During this period nine first lactation cows fed thyroprotein had an average body weight decrease of 65 lb. and a TDN intake that averaged 100 per cent of requirement. Four comparable control cows lost 10 lb. during this period with the same average TDN intake. Eleven first lactation cows receiving thyroprotein consumed TDN at 116 per cent of requirement and had a decrease of 24 lb. in body weight. Four comparable control cows consumed an average of 115 per cent in TDN but had a gain of 94 lb. during this period.

There were 48 lactations in which both extra TDN and thyroprotein were given, and in these lactations there was an average decrease of 29 lb. in the cows' body weight during this period with an average TDN intake of 117 per cent. In 18 lactations of comparable control cows there was an average gain in body weight of 37 lb. with the TDN intake averaging only 111 per cent. Thus the body weight decrease of cows fed thyroprotein was larger than that of cows not fed thyroprotein when both groups consumed TDN at approximately the same rate.

It has been observed previously that the body weight of cows showed an abrupt increase after the withdrawal of thyroprotein (22). The body weights of cows before and after the withdrawal of thyroprotein were compared with the body weights over a similar period for the control group of cows. In 48 lactations the thyroprotein-fed cows gained an average of 4.1 lb. per day for the 18 days following the removal of thyroprotein. The control cows in 24 lactations had an average increase of only 1.0 lb. per day. In this experiment the amount of TDN fed usually was decreased when thyroprotein was removed from the ration.

The large decrease in body weight when thyroprotein was fed and the rapid increase when thyroprotein was withdrawn are in harmony with the evidence that there is a difference in the water metabolism and the amount of body water between hyperthyroid and normal cows (6).

An estimate of the growth of cows during the experiment was made by comparing the average body weights during the first 50 days of successive lactations. Seven control cows after their second calving averaged 118 per cent of their first lactation body weight but 18 cows receiving thyroprotein averaged only 112 per cent. A similar difference was noted after the third calving. The relative body weights of control and thyroprotein-fed cows in their fourth, fifth, and sixth lactations showed no differences.

The findings reported here are similar to those of previous investigations, in that body weight was found to decrease after thyroprotein was fed. The data of this experiment indicate that the extent of this decrease was dependent on the level of feed intake. It was shown also that cows fed thyroprotein and extra TDN (at the rates used in this experiment) lost more body weight than did comparable cows not fed thyroprotein. The feeding of thyroprotein for approxi-

mately 300 days during the first lactation may have decreased the growth of the cows to a small degree.

Effect on the efficiency of production. The best practical expression for comparing the efficiency of conversion of food nutrients into milk is the amount of fat corrected milk produced per unit of total digestible nutrients consumed. This value has been calculated for the control and the thyroprotein-fed cows for all the lactations in which mastitis or some other factor did not invalidate the milk record. Only the period from the 50th to the 305th day of lactation was used to make this comparison. These values are presented in the top portion of Table 4. In this comparison the thyroprotein-fed cows were only silghtly less efficient than the cows in the control group for the first lactation, but the thyroprotein-fed cows were less efficient than normal cows in the second to fifth lactations, inclusive.

These values do not include any consideration for the differences in body weight changes that were found between the two groups. The values for the efficiency of milk production when body weight changes were given the usual consideration (13) are presented in the lower portion of Table 4. During the first lactation the values for seven control cows averaged 1.66 with a standard deviation of $\pm$ 0.04 lb. FCM and for the 20 thyroprotein-fed cows 1.49 $\pm$ 0.21 lb. per pound of net TDN consumed. The difference between the two values was

TABLE 4

Output-input ratios for control cows and thyroprotein-fed cows from the 50th to the 305th day of successive lactations

Group	No. of cows	Lactation					
		1st	2nd	3rd	4th	5th	6th
		Lb. FCM produced/lb. TDN					
Control	7	1.49	.....	.....	.....	.....	.....
Thyroprotein	20	1.43	.....	.....	.....	.....	.....
Control	6	1.50	1.46	.....	.....	.....	.....
Thyroprotein	15	1.47	1.32	.....	.....	.....	.....
Control	5	1.52	1.48	1.50	.....	.....	.....
Thyroprotein	7	1.58	1.32	1.36	.....	.....	.....
Control	2	1.70	1.59	1.63	1.65	1.56	1.55[b]
Thyroprotein	3	1.61	1.39	1.44	1.44	1.43	1.63
		Lb. FCM produced/lb. TDN [a]					
Control	7	1.66	.....	.....	.....	.....	.....
Thyroprotein	20	1.49	.....	.....	.....	.....	.....
Control	6	1.67	1.60	.....	.....	.....	.....
Thyroprotein	15	1.54	1.35	.....	.....	.....	.....
Control	5	1.67	1.61	1.60	.....	.....	.....
Thyroprotein	7	1.69	1.39	1.44	.....	.....	.....
Control	2	1.70	1.68	1.70	1.68	1.66	1.63[b]
Thyroprotein	3	1.67	1.37	1.49	1.47	1.41	1.55

[a] Excluding TDN necessary for the body weight changes (gross efficiency), that is, subtracting 3.53 lb. TDN per lb. of weight gain and adding 2.73 lb. TDN/lb. of loss in weight to the TDN consumed during the entire period.

[b] One cow only in 6th lactation of control cows.

significant ($P = 0.05$). With the rapid body weight changes and probable shift in water balance, the use of the usual TDN factors for body weight change may overestimate the caloric value of the weight decrease. The use of these factors introduces only a small error in long-term experiments.

During the second, third, fourth, fifth, and sixth lactations all the cows receiving thyroprotein, with one exception, were less efficient than they were during their first lactations. All control cows maintained practically the same efficiency throughout each of the successive lactations. The difference between efficiency during the first and the second and/or the third lactations for the cows fed thyroprotein was significant ($P < 0.02$). This was true for the data presented in the upper and lower portions of Table 4. The average of 1.67 ± 0.16 lb. FCM per pound of TDN for three cows during their first lactation was more than the average of 1.46 ± 0.16 lb. during their second to sixth lactations inclusive ($P = 0.05$).

The reason for the decreased efficiency during the later lactations was not evident. The level of production and fat test in the later lactations were below expected levels, and undoubtedly this was a large factor in the decreased efficiency during the later lactations.

Effect on breeding performance. The total number of services per conception for both groups of cows was tabulated according to breed and is presented in Table 5. Both groups of Jersey cows had a normal value for services per conception, although two cows in the thyroprotein group were not pregnant at 168 and 290 days in their sixth lactation when they were slaughtered. For no apparent reason, abnormally high value was observed for the cows in the Holstein control group of this experiment. The first group of Holstein cows fed thyroprotein had a very high value for the number of services per conception when they were heifers. After being fed thyroprotein for one or two lactations they failed to conceive and were slaughtered. The second group of Holstein cows had a normal conception rate during the first and second lactations when thyroprotein was fed and then were slaughtered or transferred to other experiments.

The data on the breeding history of these cows indicates that the thyroprotein had no damaging effect on the breeding performance of normal cows when fed for extended periods, as in this experiment. It is possible that the adverse effect noted on the first group of Holstein cows fed thyroprotein was aggravated by

TABLE 5

Services per conception for the cows while on this experiment and when they were heifers

Breed	Treatment	No. of cows	Services per conception	
			As heifers	While on experiment
Jersey	Control	3	1.6	1.6
Jersey	Thyroprotein	9	1.2	1.6
Holstein	Control	6	1.4	3.7
Holstein	Thyroprotein [a]	5	3.4	6.4
Holstein	Thyroprotein [b]	5	.....[b]	1.8

[a] Group that originated at Beltsville and were slaughtered because of infertility.
[b] Group that originated in 2 other herds and were pasture-bred as heifers.

some congenital anomaly. Since the two sets of cows reacted differently, it is possible that further data on the effect of thyroprotein feeding on breeding performance are needed. Four of the five cows in the first group showed symptoms of nymphomania over portions of the period when thyroprotein was fed. Thyroprotein was withdrawn from one of these cows for a period of 150 days after the completion of the second lactation. This cow did not conceive during this period or change in her nymphomaniac tendencies.

After thyroprotein was fed, it was noticed that there was a temporary cessation of estrus in some of the cows. The interval from the time thyroprotein feeding was initiated until the next estrus period was calculated. In 58 lactations of cows receiving thyroprotein this interval averaged 48 days with a standard deviation of $\pm$ 38. A similar calculation for the control cows averaged 21 $\pm$ 21 days. This difference between the two groups was significant ($P > 0.01$). The interval from calving to the first estrus period averaged 47 days for both groups of cows. The average interval from calving to conception for 43 lactations of cows fed thyroprotein was 142 days, and for 23 lactations of control cows it was 155. In all cases no attempt was made to achieve conception until 90-100 days after the previous calving date.

The feeding of thyroprotein to open cows apparently caused a delay in the appearance of the next estrus period but it had no effect on calving interval.

Effect on calves born to cows fed thyroprotein. A total of 44 calves was born to cows that received thyroprotein in this experiment. During the last 90 days of the gestation period no thyroprotein was fed. All calves born to these cows

TABLE 6

Average birth weight and mortality rate of calves in the nutrition herd at Beltsville, 1945-52

Breed and sex of calves	Calves born alive	Average birth weight	Calves that died during 1st mo.
	(No.)	*(lb.)*	*(%)*
From dams fed control rations			
Jerseys:			
Females	109	52	15.0
Males	118	56	18.0
Holsteins:			
Females	52	89	4.0
Males	65	94	12.0
Av. loss			14.0 [a]
From dams fed thyroprotein during previous lactation			
Jerseys:			
Females	16	55	50.0
Males	18	57	28.0
Holsteins:			
Females	3	88	0.0
Males	7	103	29.0
Av. loss			36.0 [b]

[a] Does not include 23 (6%) stillbirths mainly from 1st calf heifers. There were no stillbirths from thyroprotein cows.

[b] Does not include 3 abortions. Number of abortions from normal cows was not calculated.

appeared normal at birth, and the cows had no unusual amount of calving difficulties at parturition. The birth weight of the calves was normal for the respective breeds and sexes, as indicated in Table 6.

The mortality rate of the calves from cows fed thyroprotein was higher than normal. The mortality rate to 30 days of age was 36 per cent, compared to 14 per cent for all other calves born alive in this herd over the same period of time (Table 6). The reason for the higher mortality rate during early calfhood was not evident. Most of the calves that died developed diarrhea at an early age and did not respond favorably to the usual therapeutic practices that were the same for all the calves. Autopsies were always performed, and nothing abnormal except enteritis, gastro-enteritis, and occasionally some lung involvement was found.

A study of the activity of the thyroid gland of these young calves was not made. The thyroid weight of seven of the calves that died at 2 to 15 days of age averaged 91 mg. per pound of body weight. Six calves that died at 1 to 10 days of age averaged 109 mg. of thyroid tissue per pound of body weight.

Effect on blood constituents. The concentration of several blood constituents was determined at infrequent intervals in many of the cows. The hemoglobin values ranged from 8 to 12 g. per 100 ml. blood for both control and thyroprotein groups. The same range was found for the latter group when not receiving thyroprotein. Hematocrit values also showed a wide range (29 to 43 per cent). Sedimentation rates were normal except for the blood of one thyroprotein-fed cow which always had an abnormally fast sedimentation rate.

Serum phosphatase concentrations showed no consistent change when thyroprotein feeding was either initiated or discontinued. It both increased and decreased when each of these changes was made. The values ranged from 1 to 12 Bodansky units per 100 ml. of serum with large cow-to-cow variation and only small changes throughout the lactation period. The inorganic phosphorus concentration of the serum (or the plasma) ranged from 3.5 to 8.0 mg. per cent in both groups of cows. The plasma calcium concentration ranged from 9 to 13 mg. per cent in both groups and showed no change when the thyroprotein feeding was initiated or withdrawn.

A few determinations for plasma vitamin A and carotene were made. A total saponification procedure followed by chromatographing in order to separate the carotene from the vitamin A was used. The true plasma vitamin A ranged from 13 to 24 γ per 100 ml., averaging 20.5, for 7 cows receiving thyroprotein and from 19 to 35 γ per 100 ml., averaging 23.7, for 7 normal cows. One cow in the thyroprotein group had a plasma value of 7.0 γ vitamin A per 100 ml. one week after being sick, off-feed, and aborting. The true carotene values ranged from 116 to 326 γ per 100 ml. plasma for the cows in the thyroprotein group and from 190 to 378 for cows in the control group.

Post-mortem findings. During this experiment several of the cows were slaughtered. An autopsy was performed in each case, and in most of the cows the weights of several organs were obtained.

The four Holstein cows that did not conceive had very small ovaries, which were atrophic and contained some deeply imbedded corpus luteum-like tissue. The examination record of the veterinarian indicated that these four animals had very small ovaries when they were heifers. It is possible that these cows had some congenital abnormality which caused them to be infertile when the thyroprotein was fed.

The sizes of the thyroid glands and the ovaries of the cows receiving thyroprotein were below those of normal cows. The pituitary and adrenal glands and the kidneys tended to be slightly smaller than the average, according to the data of Swett *et al.* (20). The weights of the hearts of cows fed thyroprotein were above that for the average cow.

Under the influence of thyroprotein the thyroid gland would be expected to be decreased in size. This was found to be true for the cows in this experiment. Also, the thyroid glands were lighter in color than those in normal cows. The relative weights of other organs were only slightly different than those of normal cows, but with the small numbers observed no great significance can be given to these observations.

At the time of slaughter, some of the ribs and metacarpal bones were obtained from these cows. The lower (distal) 10 per cent of the rib was taken for a determination of its ash content. Three ribs from two control cows averaged 56.7 per cent ash on the anhydrous-fat-free basis. Twenty-two ribs from nine cows in the thyroprotein group averaged 56.3 per cent. An X-ray examination of the metacarpals and some of the ribs showed no differences in bones from the control and the thyroprotein group. Apparently no detectable decalcification had occurred in the bones of the cows fed thyroprotein.

SUMMARY

An experiment was carried out in which thyroprotein was fed for approximately 300 days of each lactation for the length of time that the cows remained in the herd. Twenty cows were started in their first lactation, and three completed their sixth lactation in the group that received thyroprotein. Control cows were maintained under identical conditions. No evidence of early senescence or increased rate of disposal or turnover was found in the cows that received thyroprotein.

Thyroprotein caused an immediate increase in the level of milk production and in butterfat test in practically all animals. The feeding of extra nutrients to a level of 125 per cent of Morrison's maximum TDN requirement was found to help sustain these increases. Without the feeding of the extra nutrients the increases in fat test and milk production were only transient. With the feeding of extra nutrients the increase in fat test was noticeable for only about 2 months. The feeding of thyroprotein and extra nutrients did not result in a greater amount of milk produced over an entire lactation than in comparable cows fed extra nutrients only. The fat test and the mature equivalent FCM production of cows fed thyroprotein through several lactations were lower during the second and later lactations than the corresponding values for the first lactations.

Body weight decreased more in the cows fed thyroprotein than it did in the cows not fed thyroprotein at equivalent levels of TDN intake. A very rapid increase in body weight occurred immediately after the withdrawal of thyroprotein.

If one disregards the difference in body weight changes, the efficiency of conversion of TDN into milk by the cows fed thyroprotein was practically the same as that by normal cows for the first lactation. The efficiency was less than the value for normal cows in later lactations. When the usual TDN allowance was made for body weight changes, the cows receiving thyroprotein were less efficient than normal cows during their first lactation. With either method of calculation the efficiency of cows fed thyroprotein decreased in subsequent lactations but remained constant in the control cows.

The mortality rate of calves born to cows fed thyroprotein under the conditions of this experiment was higher than for other calves. Observations on other aspects of this study also are mentioned.

The evidence presented has demonstrated that it is not a desirable practice to feed thyroprotein to dairy cattle during the greater portion of the declining phase of lactations, especially during successive lactations. The adverse effects, such as decrease in efficiency of production, increase in mortality of calves, and decreased rate of milk and fat production in subsequent lactations, that were noted in this experiment might not occur or be of significance if thyroprotein were fed for short periods of time. If this is true, then the factor of importance is the efficiency of conversion of food nutrients into milk. Data on the economic aspects and efficiency of feeding thyroprotein to cows under short-term practical conditions are not available. This type of information is necessary in order to evaluate properly the use of thyroprotein preparations on a practical short-term basis. This problem is now under investigation.

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A COMPARISON OF HOMOGENIZED WHOLE MILK AND EGG YOLK-PHOSPHATE AS DILUTERS FOR BULL SEMEN

W. H. DREHER AND J. H. WEBB
Badger Breeders Cooperative, Shawano, Wisconsin

The use of milk as a diluter for bull semen was reported by Thacker and Almquist (5) in 1951. They suggested that the non-return rate from its use in artificial insemination was equal to, if not slightly better than, that from the use of yolk-citrate. Almquist and Thacker (1) report that the increases in six low-fertility bulls accounted for the major portion of the improvement obtained with milk plus antibiotics as compared to yolk-citrate plus antibiotics.

The present study was an attempt to confirm the reported results and to compare milk and yolk-phosphate for each of two breeds of bulls, for the different bulls of each breed, and for each of two different months of the year.

MATERIALS AND METHODS

The experiment was run in December of 1951 and May of 1952. These two months have, in the history of the bull stud, been months of high and low fertility, respectively. The average 60-90 day non-return rates for Holstein bulls over a 6-year period have been 68.4 per cent for the months of September, October, November, and December and 63.7 per cent for the months of May, June, July, and August. The Guernseys have averaged 64.1 and 58.6 per cent for the two periods, respectively. Portions from the same ejaculate of semen from each of the various Holstein and Guernsey bulls were diluted with the two different diluters and used by some of the technicians of the organization. Alternate cows to be bred to a given bull were inseminated with the milk-diluted semen and with the yolk-phosphate. Areas furnishing approximately 22 per cent of the first service cows for the month were included in the experiment in December and 32 per cent in May. Non-return results were obtained also on 15,013 cows outside the experiment in December and 18,535 in May from semen of the same bulls, using yolk-phosphate. Data on these inseminations are used as an independent estimate of the relative fertility of the different bulls within the breed.

Homogenized whole milk was used for the diluter. This was boiled at a temperature of 98.5° C. for 20 minutes in a 3,000 cc. Erlenmeyer flask. Following the boiling process, the milk was filtered through a Pyrex funnel containing a pledget of sterile glass wool. After the milk was cooled, 1,000 units of penicillin and 1 mg. of streptomycin were added per milliliter. The dilution rate was 1:50. The yolk-phosphate combination was the standard 1:2 mixture with the same antibiotics added and the same dilution rate as for the milk. The statistical tests of significance of differences in non-return rates were made by the chi-square test for independence (4). Examination of the differences between the two

Received for publication December 18, 1952.

diluters in the two different breeds or in the two different months or in the different bulls of each of the breeds was made by angular transformation of the non-return rates and analysis of variance (3).

RESULTS

The differences between the two diluters were statistically highly significant for the Holsteins in each month (Table 1), the actual difference being of the order of 8 per cent in December and 9 per cent in May in favor of the boiled milk. In the Guernsey breed (Table 2) there were differences of 5 per cent and 18 per cent in December and May, respectively, ($P = 0.07; 0.01$) in favor of the boiled milk. Examination next was made to see whether the differences between the two diluters were in themselves different between the two breeds. The 8 per cent for Holsteins and 5 per cent for Guernseys in December did not differ significantly ($P = 0.3\text{-}0.5$). However, the two breed differences in May, 9 per cent for Holsteins and 18 per cent for Guernseys, did differ significantly ($P = 0.01$). There was a 13 per cent drop in non-return rate for Guernseys on yolk-phosphate between December and May (from 62.8 per cent to 49.8 per cent: 60-90 day rate of non-return; $P = 0.01$). This is in marked contrast to the milk diluter, for

TABLE 1

Comparison of milk and yolk-phosphate diluted Holstein semen

	December						May					
		Exp. milk		Exp. yolk-phosphate				Exp. milk		Exp. yolk-phosphate		
Bull	Non-exp. yolk N.R.%	No. 1st service	N.R.%	No. 1st service	N.R.%	Exp. diff.	Non-exp. yolk-phosphate N.R.%	No. 1st service	N.R.%	No. 1st service	N.R.%	Exp. diff.
H20	58.3	34	85.3	23	65.2	20.1	68.7	72	75.0	61	49.2	25.8
H36	64.2	23	82.6	31	54.8	27.8	53.4	107	68.2	100	45.0	23.2
H38	72.3	56	76.8	56	69.6	7.2	67.6	140	69.3	93	73.1	−3.8
H39	66.7	20	90.0	21	71.4	18.6	.....	.....	.....	.....	.....	.....
H40	60.3	46	76.1	48	68.8	7.3	58.9	106	67.9	85	61.2	6.7
H41	58.4	97	72.2	76	60.5	11.7	58.1	54	74.1	54	55.6	18.5
H42	65.3	26	69.2	27	63.0	6.2	62.9	173	71.1	168	66.8	4.4
H44	66.5	46	73.9	45	73.3	0.6	69.4	119	82.4	95	71.6	10.8
H46	51.1	52	78.9	36	41.7	37.2	29.4	33	75.8	26	46.2	29.6
H47	.....	.....	.....	.....	.....	.....	47.7	161	64.0	152	50.7	13.3
H48	70.0	30	80.0	39	79.5	0.5	65.7	159	76.7	163	67.5	9.2
H49	69.0	85	80.0	76	68.4	11.6	65.4	118	72.0	100	61.0	11.0
H51	61.7	176	59.7	168	62.5	−2.8	63.0	182	68.7	148	72.3	−3.6
H53	.....	.....	.....	.....	.....	.....	45.6	59	54.2	62	48.4	5.8
H54	61.9	95	73.7	73	68.5	5.2	56.6	151	66.9	150	52.0	14.9
H55	65.1	102	76.5	76	68.4	8.1	62.9	234	73.1	218	65.6	7.5
H57	65.8	55	69.1	59	64.4	4.7	58.7	113	69.0	89	58.4	10.6
H58	66.4	93	78.5	108	62.0	16.5	69.2	162	68.5	157	73.2	−4.7
H59	66.5	136	66.2	130	66.2	0.0	.....	.....	.....	.....	.....	.....
H60	67.1	65	72.3	36	63.9	8.4	54.3	202	75.7	187	61.5	14.2
H61	61.1	179	68.7	177	66.1	2.6	63.1	134	68.7	131	58.8	9.9
H62	63.8	99	79.8	87	58.6	21.2	42.9	40	80.0	39	59.0	21.0
	63.7[a]	1515[b]	72.7[a]	1392[b]	64.8[a]	7.9[a]	60.8[a]	2868[b]	70.1[a]	2606[b]	60.9[a]	9.2[a]

[a] Average.
[b] Total.

TABLE 2

Comparison of milk and yolk-phosphate diluted Guernsey semen

	December						May					
		Exp. milk		Exp. yolk-phosphate			Non-exp. yolk-phosphate	Exp. milk		Exp. yolk-phosphate		
Bull	Non-exp. yolk N.R.%	No. 1st service	N.R.%	No. 1st service	N.R.%	Exp. diff.	N.R.%	No. 1st service	N.R.%	No. 1st service	N.R.%	Exp. diff.
G36	53.3	23	65.2	23	56.5	8.7	43.0	69	69.6	69	42.0	27.6
G47	65.1	65	81.5	67	67.2	14.3	59.1	116	72.4	118	62.7	9.7
G53	65.1	15	53.3	14	78.6	−25.3	.....	.....	.....	.....	.....	.....
G55	56.5	30	63.3	34	61.8	1.5	.....	.....	.....	.....	.....	.....
G59	46.8	46	65.2	49	49.0	16.2	52.6	108	68.5	96	34.4	34.1
G61	.....	.....	.....	.....	.....	.....	65.7	127	78.7	103	66.0	12.7
G67	66.7	60	71.7	54	72.2	−0.5	59.4	154	79.2	127	56.7	22.5
G69	53.8	48	62.5	41	51.2	11.3	.....	.....	.....	.....	.....	.....
G70	60.1	37	73.0	29	51.7	21.3	51.2	179	62.0	152	49.3	12.7
G71	61.2	144	67.4	121	71.9	−4.5	.....	.....	.....	.....	.....	.....
G72	52.0	56	67.9	59	49.2	18.7	45.1	108	63.9	92	52.2	11.7
G73	54.1	60	65.0	47	61.7	3.3	50.6	107	70.1	94	58.5	11.6
G74	54.6	50	64.0	48	64.6	−0.6	54.5	111	63.1	97	51.5	11.6
G75	70.1	49	65.3	54	68.5	−3.2	66.8	161	71.4	123	62.6	8.8
G76	.....	.....	.....	.....	.....	.....	51.4	154	69.9	125	40.8	29.1
G77	.....	.....	.....	.....	.....	.....	51.6	132	59.1	115	35.7	23.4
	59.1[a]	683[b]	67.8[a]	640[b]	62.8[a]	5.0[a]	53.7[a]	1743[b]	67.8[a]	1422[b]	49.8[a]	18.0[a]

[a] Average.
[b] Total.

which the percentage of 60-90 day non-return was 67.8 in both months. The 60-90 day non-return rates for the Holsteins on yolk-phosphate were 64.8 and 60.9 for December and May, respectively, and for the milk diluter, 72.7 and 70.1. The May results were slightly but statistically insignificantly lower than in December for each diluter ($P = 0.5$-0.7).

These results suggest certain peculiarities about both the breed difference and the diluter difference in this experiment. The two breeds differed insignificantly in each month on the milk diluter. Their difference is also insignificant on the yolk-phosphate diluter in December. The difference between the Holsteins and Guernseys on the latter diluter is very pronounced in May (11.1 per cent; $P = 0.01$). The two breeds for the stud as a whole over a 6-year period have shown differences ranging from 2.8 to 6.7 for the various months, with an average difference of 4.7. No breed difference in seasonal variability has been noted. It seems possible that the Guernseys in this particular trial were more sensitive to some seasonal factor than were the Holsteins. Further, the peculiar properties of milk which make it valuable as a diluter were less influenced by season than were the properties in egg yolk and were better able to prevent the breed difference in the two seasons in this experiment.

The advantage of milk over yolk-phosphate might occur fairly uniformly with all bulls or it might be particularly advantageous for the dilution of semen from certain bulls only. The differences between the two diluters were examined for their uniformity from one bull to another. Statistical tests showed the differences from one bull to another to be insignificant in both breeds in

December. In May, however, there was a much greater indication of bull differences in the degree to which milk excelled yolk-phosphate as a diluter ($P = 0.01$-0.02, Holsteins; $P = 0.10$, Guernseys).

The samples from bulls in use in the 2 months were not identical. Eighteen of the Holstein bulls and nine of the Guernsey bulls were used in both months. Other bulls were used in only one. The degree to which bulls repeat themselves in showing a certain degree of advantage for the milk diluter over yolk-phosphate constitutes related evidence on the existence of bull differences in their responses to these diluters. The correlation for the Holstein breed between the differences between these two diluters in December and comparable differences in May was 0.79 ($P = 0.01$). The correlation for the Guernsey breed was not statistically significant ($r = 0.15$). The initial difference between bulls for the Holsteins in December, although not statistically significant, would appear in general to be in the same direction as they were in May. For the Guernseys, no evidence was obtained for the repeatability even of insignificant differences between bulls.

A better characterization of differences between bulls in level of fertility was available from the non-experimental inseminations made concurrent with the experiment than from the experiment itself. Rating of the bulls on that basis had the advantage of being an independent estimate and at the same time being based on a larger body of data. Correlations were calculated between the differences in non-return rate on milk and yolk-phosphate and the non-return rates on the same bulls when their semen was diluted with yolk-phosphate and used in the non-experimental breeding operation of the stud. These correlations were all negative and quite similar. They were -0.45 and -0.54 for the Holsteins and -0.48 and -0.35 for the Guernseys for the months of December and May, respectively. The over-all correlation of -0.45 was highly significant. Bulls that were lowest in their non-return rate on yolk-phosphate were the ones that tended to give the greatest added response when their semen was diluted with milk.

The greater improvement apparent in bulls of low non-return rates raises the question as to mechanism, that is, whether the improvement is brought about by increased fertilization rate or by decreased embryonic death rate. Work of Kidder *et al.* (2) has suggested that the difference in performance between the bulls of highest and of lowest non-return rate in general use in artificial insemination in Wisconsin can be accounted for on the basis of a difference in fertilization rate (the difference being of the order of 20 per cent in favor of the high bulls). Thus, it might be suspected that the greater improvement in non-return rate of low bulls on milk diluter comes about by an increase in their fertilization capacity, whereas the fertilization rate of high bulls is already high enough to leave little room for improvement.

SUMMARY

A comparison has been made of yolk-phosphate and milk-diluted semen from 16 Guernsey and 22 Holstein bulls in December and in May. In previous years,

these have been months of relatively high and low fertility, respectively. Non-return rates on milk exceeded those on yolk-phosphate by 7.9 and 9.2 per cent for the Holsteins in the two months and by 5.0 and 18.0 per cent for the Guernseys. The two breeds did not differ significantly on yolk-phosphate in December or on milk in either month; in May the Guernseys were lower by 11.1 per cent than the Holsteins on yolk-phosphate.

A total of 6,009 experimental first services were made with milk-diluted semen, resulting in a 69.8 per cent 60-90 day non-return, compared to a total of 6,060 experimental first services with yolk-phosphate diluted semen, which resulted in a 59.2 per cent 60-90 day non-return. Bulls of both breeds that tended to be lowest in non-return rates on yolk-phosphate were the ones that tended to give the greatest added response when their semen was diluted with milk.

ACKNOWLEDGMENT

The authors wish to acknowledge the aid of H. E. Kidder and L. E. Casida of the Department of Genetics, University of Wisconsin, in the statistical analyses of these data and in the preparation of this paper.

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PRODUCTION CHARACTERISTICS OF CROSSBRED, BACKCROSS, AND PUREBRED RED SINDHI CATTLE IN THE GANGETIC PLAINS REGION

H. H. STONAKER [1]
Department of Animal Husbandry, Colorado A. and M. College, Fort Collins

AND

O. P. AGARWALA AND D. SUNDARESAN
Allahabad Agricultural Institute, Allahabad, India

The Allahabad Agricultural Institute (India) has long been engaged in breeding cattle for dairy purposes. The Institute is located in the Gangetic plains region, an area of great climatic stress due to variations from desert-like heat during April, May, and June, when day temperatures may reach 118° F., through the humid but less hot monsoon period of July, August, and September, to the dry, stimulating winters, which rather closely resemble summers in the high valleys of the Rocky Mountains. It is generally believed, although precise information in this specific area is not available, that the specialized dairy breeds of Europe do not produce or survive in this area as well as do indigenous cattle. The primary interest in the villages has been on bullock draft power, and cows are maintained to produce bullocks. The buffalo is more highly regarded in this region as a milk animal and not infrequently produces twice as much as the cow. City dairies commonly may pay two to three times as much for a buffalo as for a cow. However, because in addition to the draft power of cattle there is religious encouragement in their maintenance, agricultural college and governmental personnel have been particularly interested in improving dairy qualities of the Indian and Pakistan breeds. This improvement is attempted by selection within the Indian breeds and in some dairies by crossing with European dairy bulls. The immediate increased productivity of cows sired by European dairy bulls has been known in India for many years. The "Taylor breed," an interbreeding population of cattle near Patna (Bihar), is said to have been derived from crosses of European bulls and native cattle as early as 1875 (Henderson, 2). Descendants of these crosses still are considered to be good producers, and Patna villagers have high regard for the milking qualities of these nonhumped cattle. Crossbreeding of Western with Indian cattle has been widely practiced on Indian military farms. This has been done at least since 1900, and records of production on these farms indicate that one-half to five-eighths Holstein breeding gives maximum production (5). This is substantiated by Tandon (13) in a more refined analysis of the data. Thus, it seems to be well established that introduction of European dairy sires has increased milk production in India over that obtained from the locally developed cattle, and it also appears that in other tropical areas the introduction of Indian breeding may increase productivity over that of the

Received for publication January 5, 1953.

[1] Fulbright lecturer on sabbatical leave.

European breeds. Experiences in Jamaica (Howe, 3) indicated that Zebu crosses yielded considerably more than purebred Jerseys, Guernseys, or grades with less than half Zebu. In the United States recent experiments indicate that under Maryland conditions Jersey × Red Sindhi crosses may yield slightly more butterfat than do pure Jerseys (Fohrman, 1).

Practically ever since its establishment in 1910, the Allahabad Institute has been confronted with the problem of breeding profitable milk animals. Various breeds have been tried, such as Red Sindhi, Gir, Hariana, Sahiwal, and Kankrej. Crosses of these with Holsteins, Guernseys, Brown Swiss, and Jerseys have been made. Impressions of men who were in charge of the herd during the early years have been recorded by Schneider (10), Rathore (8), Tandon (12), and Saxena (9). Although detailed results with various breeds and crosses have not been reported, the observations of the men in charge led them to favor the Red Sindhi breed. The breeding program which has been followed was an adaptation of suggestions by Hutchinson and Joshi (4) as outlined by Schneider (10). The plan was to hold the comparatively high production of the crossbred cows and yet avoid the complications of continual intercrossing between the Jersey and Red Sindhi breeds. This was to be done by backcrossing to the Red Sindhi and simultaneously selecting for high production. The higher producing Red Sindhi cows were bred to Red Sindhi bulls, and the lower producing Red Sindhi cows were bred to Jersey bulls. The crossbreds then were backcrossed to Red Sindhi sires. This breeding program has been in effect since 1934. About the same time the record keeping was systematized so that the informational value of the herd was greatly increased.[2]

Rathore (8) reported on differences in growth of the Red Sindhis and their crosses. He found crossbred females exceeded pure Red Sindhis by 12 per cent in body weight at 2 years of age but by only 1 per cent in wither height. Tandon (12) in a study of gestation time of Red Sindhis and crosses found that the F_1 Jersey × Red Sindhi calves were born after a gestation period 5.8 days shorter than that required for the Red Sindhi calves. Production per lactation for all lactations in this herd of Red Sindhis, crossbreds, and backcrosses was reported in the Indian Ministry of Agriculture (5). Although changing environmental effects were not considered in records being made by different breeding groups at different times, records for the crossbreds were approximately 47 per cent higher than those for the pure Red Sindhis, and the various backcrosses generally decreased in production as relationship to the Red Sindhi increased.

ANALYSIS OF THE DATA

In the present study, production characteristics of Red Sindhi, F_1 Jersey × Red Sindhi, and first, second, and third backcrosses to the Red Sindhi were analyzed. A few data were available also on F_1 Brown Swiss × Red Sindhi and on $\frac{3}{4}$ Jersey × $\frac{1}{4}$ Red Sindhi crosses. With the system of continual backcrossing,

[2] The establishment of the breeding plan was the work of Burch Schneider and N. R. Joshi, and the original policy has been continued by N. R. Joshi, T. W. Millen, and J. N. Warner.

periodically the breeding of the herd differed considerably. Thus, there was not a random distribution of each of the breeding groups such as F_1's, first, second, and third backcrosses during the different years. Because of this correlation between time and the genetic structure of the breeding herd, it was decided to follow a system of analysis whereby the first lactation records of each breeding group were compared with first lactation records of pure Red Sindhis made in the same year. First lactation records were chosen in order to avoid the possibility that differential culling in the groups might have taken place in subsequent lactations. Another assurance that first records would be relatively free from selection bias was the fact that there was very little culling of heifers in the herd. However, a certain bias in comparing the crosses with Red Sindhis was introduced because the lower producing Red Sindhi cows were bred to Jersey bulls and the better Sindhi cows to the Sindhi bulls. Thus, there was probably a genetic bias in the present analysis in favor of the Red Sindhis. Another limitation in the data came from the fact that only three Jersey bulls sired crossbred progeny; thus, the sample of Jerseys is particularly limited.

Although all crosses could not be compared at a similar time, it was possible to check each breeding group against contemporary first lactation Red Sindhis. The comparisons were made using procedures of the R $\times$ 2 tables described by Snedecor (11). Means and differences between means were weighted by this method for the various characteristics studied. Significances of differences were computed, using the same techniques of analysis.

305-day butterfat yields. Production of the cows in different breeding groups was measured from the total butterfat yields in 305 days of the first lactation. Fat yield was used in preference to milk yield in order to eliminate the possibility

TABLE 1

First lactation 305-day butterfat yields of crossbreds, backcrosses, and Red Sindhis

Cross	No.	Weighted av. yield *(lb.)*	Check group	No.	Weighted av. yield *(lb.)*	Per cent of check group	Significance[a]
1/2 B. Swiss	11	220	Red Sindhi	22	143	153	**
3/4 Jersey	7	117	Red Sindhi	21	114	102	NS
1/2 Jersey	25	213	Red Sindhi	40	145	147	**
1/4 Jersey	68	162	Red Sindhi	63	130	124	*
1/8 Jersey	25	136	Red Sindhi	46	128	106	NS
1/16 Jersey	8	154	Red Sindhi	32	127	121	NS
1/2 Jersey	27	212	1/4 Jersey	35	172	124	*
1/4 Jersey	50	162	1/8 Jersey	25	135	120	NS
1/8 Jersey	17	156	1/16 Jersey	8	157	100	NS

[a] * Significant at 0.01 level; ** significant at 0.05 level; NS – nonsignificant.

that milk output should be corrected for the energy value. Generally in India, either daily milk yield or milk yield in the entire lactation is the record used. For cows which have very short lactations, daily milk yield would tend to be deceiving. Likewise, total amount of milk produced in an unusually long lactation would tend to favor cows with long calving intervals. At the same time, it seemed to be accepted in India that annual calving is a desired objective (Joshi,

Joshi, and Warner, 6); so it appeared that the 305-day lactation record was a more accurate measure of desired production characteristics than either daily yield or yield in the total lactation period.

The data in Table 1 on butterfat production behave in a pattern similar to that found in other Indian herds (Tandon, 13), in that the crossbreds showed great superiority over the pure Indian breed and that backcrossing to either breed tends to have an adverse effect on production. The association appears clear cut except for the unexpected high production of the few 1/16 Jerseys. Checking among the crosses, however, did not bear out this evidence of superiority of the 1/16 cross. Thus, it would appear that on a first lactation basis the production of animals with 1/8 Jersey or less will be only slightly higher than pure Red Sindhis. This would substantiate experimental evidence in other countries that the production of grade cattle is approximately the same as the purebreds after two to four backcrosses.

In Figure 1 the performances of first lactation Red Sindhis and crossbreds are shown by years.

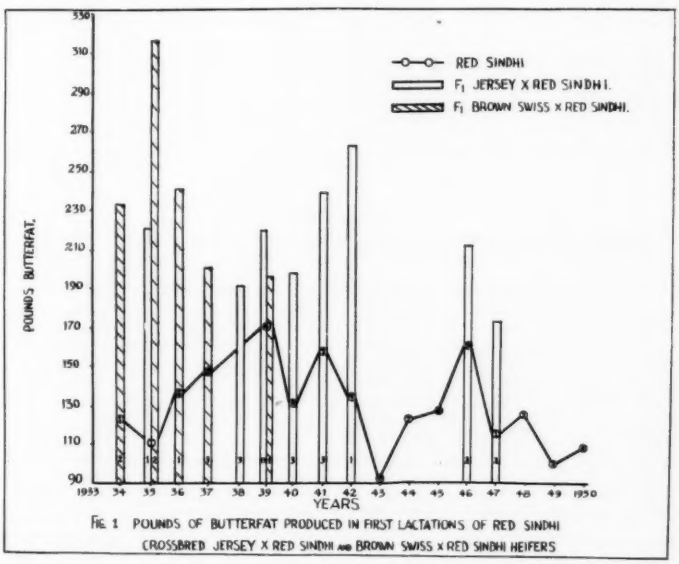


FIG. 1 POUNDS OF BUTTERFAT PRODUCED IN FIRST LACTATIONS OF RED SINDHI CROSSBRED JERSEY X RED SINDHI AND BROWN SWISS X RED SINDHI HEIFERS

Age at first calving. Table 2 indicates that there were large variations in ages of the different groups at first calving. This is particularly important, as it determines how quickly the cow comes into her productive life. Ability to calve at an early age would be considered a desirable trait. Comparisons again were made on the basis of weighted differences between the averages of heifers calving in the same year.

Age at first calving appeared to be greatly decreased by the introduction of Jersey genes. The greater the relationship to the Jersey the younger was the

comparative age at first calving. No comparisons beyond ¾ Jersey relationship were possible. Variations in the calving age of Red Sindhis in the pairs of comparisons were due to the effect of heifers calving in different years.

Calving intervals. Breeding efficiency of the various groups was evaluated by the length of time elapsing between the births of the first and second calves. Delayed calvings appear to decrease total lifetime milk production and increase generation intervals. Thus, it is a trait of economic interest. In Table 3 the interval between the first and second calves was compared for cows having their first calves in the same year.

TABLE 2

Months of age at first calving for crossbreds, backcrosses, and Red Sindhis

Cross	No.	Weighted av. *(mo.)*	Check group	No.	Weighted av. *(mo.)*	Per cent of check group	Significance
3/4 Jersey	5	31	Red Sindhi	13	47	66	**
1/2 Jersey	27	29	Red Sindhi	49	41	71	**
1/4 Jersey	77	36	Red Sindhi	71	40	90	**
1/8 Jersey	30	39	Red Sindhi	51	43	91	**
1/16 Jersey	10	41	Red Sindhi	36	47	87	NS
1/2 Jersey	27	27	1/4 Jersey	37	33	82	**
1/4 Jersey	59	39	1/8 Jersey	30	38	102	NS
1/8 Jersey	21	42	1/16 Jersey	10	40	105	NS

TABLE 3

Interval in months between first and second calves for crossbreds, backcrosses, and Red Sindhis

Cross	No.	Weighted av. *(mo.)*	Check group	No.	Weighted av. *(mo.)*	Per cent of check group	Significance
3/4 Jersey	3	12.4	Red Sindhi	6	18.2	68	*
1/2 Jersey	23	13.8	Red Sindhi	31	14.2	97	NS
1/4 Jersey	53	16.0	Red Sindhi	53	17.0	94	NS
1/8 Jersey	11	17.1	Red Sindhi	32	17.4	98	NS
1/16 Jersey	6	17.9	Red Sindhi	24	16.9	106	NS
1/2 Jersey	24	13.8	1/4 Jersey	28	14.9	92	NS
1/4 Jersey	38	16.5	1/8 Jersey	13	17.5	94	NS
1/8 Jersey	8	17.1	1/16 Jersey	5	18.0	95	NS

TABLE 4

Calving intervals (gross averages) for cows of different breeding

	Calving intervals											
	No.	1st	No.	2nd	No.	3rd	No.	4th	No.	5th	No.	6th
		(mo.)		*(mo.)*		*(mo.)*		*(mo.)*		*(mo.)*		*(mo.)*
Red Sindhi	69	16	59	15	53	14	37	13	28	14	20	15
1/2 Jersey	27	13	26	13	21	14	12	14	17	15	15	16
1/4 Jersey	56	16	36	14	28	16	16	16	9	16	5	16
1/8 Jersey	13	18	8	16	5	20	1	21	1	16	1	14
1/16 Jersey	6	18	4	16	2	16	1	14	....	....	....	....

Although numbers and differences were too small to be significant for pairs of comparisons, the consistency with which the crosses had slightly shorter calving intervals than the Red Sindhis made it appear that there was a small but probably real difference between the crosses and the Red Sindhis in this characteristic.

A less precise but more inclusive analysis included calving intervals subsequent to the first (Table 4). Its lack of precision was due to confounding of years and breeding groups. The effect of lumping all years together without considering the fact that different breeding groups made records in different years is illustrated by comparing the column on first calving interval of this table with the previous table.

Butterfat percentages. The percentage of butterfat in milk was studied also by comparing crosses with pure Red Sindhis within years. The crosses had almost the same butterfat percentages as the Red Sindhis, with a tendency to be slightly higher. Table 5 shows the average butterfat content of the various groups in their first lactations.

TABLE 5

Butterfat percentage in milk from crossbreds, backcrosses, and Red Sindhis

Cross	No.	Weighted av. (%)	Check group	No.	Weighted av. (%)	Per cent of check group	Significance
1/2 Jersey	25	4.95	Red Sindhi	34	4.86	102	NS
1/4 Jersey	74	4.82	Red Sindhi	66	4.77	101	NS
1/8 Jersey	26	4.87	Red Sindhi	46	4.71	103	NS

Relative rate of disappearance from the herd. The question of survivability as it refers to the longevity of animals was scarcely answerable in these data, since the cows had been continually subjected to culling. At times the policy was to increase the herd and at other times to decrease it. Since the production of the different breeding groups also had varied from time to time, there was difficulty in disassociating the policy concerning herd size and the amount of culling that was being done on the crosses produced at that time.

An indication of the total survival rate in the milking herd was obtained from a study of the number of animals in different breeding groups in the first, second, third, fourth, fifth, and sixth lactations. These animals had first lactations between 1937-1943; thus, they were of ages which could have had six lactations by 1950-1951.

It is difficult to draw definite conclusions from these comparisons. One difficulty is that widely differing numbers of animals were brought into the herd from year to year. It seems fairly certain that the crosses had not been culled as heavily as the Red Sindhis, and this is an indication that their production and survivability had held up well over a period of time.

Death losses among heifer calves to 6 months of age could be analyzed for the period of 1943 to 1951, but there were not enough data to remove year effects.

In these years 1/8 Jersey, 1/4 Jersey, and Red Sindhi calves were born annually. Records were not available on the F_1's born in earlier years, nor could records on bull calves be used, for these calves generally were disposed of shortly after birth. During this period the ratios of heifer calves alive and dead at 6 months of age were as follows: 1/8 Jersey — 42 alive, 28 dead; 1/4 Jersey — 14 alive, 17 dead; Red Sindhi — 41 alive, 40 dead. In comparison with the Red Sindhi group, neither the 1/4 Jersey nor the 1/8 Jersey gave significant Chi-square tests, although the latter group approached a 0.05 level.

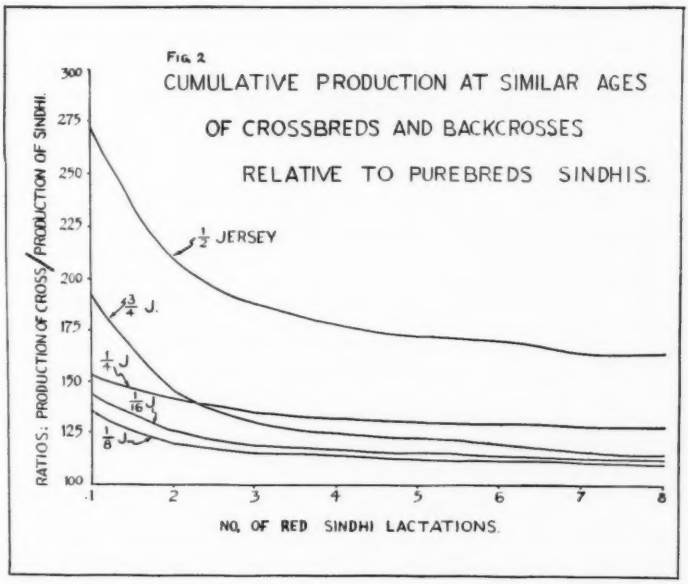
Thus, from the fragmentary evidence available on adults and young animals, there appears to be greater total survivability of animals with some Jersey breeding. It is certain that the F_1 cows have remained in the Allahabad herd much longer than the Red Sindhis. So far as viability of young stock is concerned, differences were not significant. All groups showed rather heavy death losses up to 6 months of age.

DISCUSSION

The two characteristics in which the crosses showed the greatest increases over the Red Sindhis were to a degree multiplicative in their effect on production in a given period of time. Thus, the crosses not only gave more milk per lactation but they began their productive life earlier than the Red Sindhis. In a population in which there was no intentional culling it would be possible to estimate properly total lifetime productivity of the different breeding groups. In this study, however, it was not possible to do this because of the differential culling rates in the various groups. It seemed likely that this came about largely because of differences in production of the different groups. However, if it can be assumed that the differences in the first lactations are representative of differences in later lactations, a reasonable assumption considering previous analyses of all lactations in the herd, then it is possible to make an estimate of lifetime production of the crosses and Red Sindhis. The advantage of crossbred or backcross cows over Red Sindhi cows of the same age can be expressed as the number of lactations of the crosses $(n + x)$ relative to the Red Sindhi lactations (n) multiplied by the ratio of first lactation production of the crosses (J) to the first lactation production of Red Sindhis (S), or $\frac{(n + x)}{n}\left(\frac{J}{S}\right)$. Thus, for example, at the age when Red Sindhi cows have finished their first lactations, Jersey × Red Sindhi cows have almost completed a second lactation and have given 2.72 times as much total yield. As the ages increase, the relative advantage of the cross decreases because of the diminishing effect of the earlier age at first calving. However, if one weights the advantages at different ages by the number of cows of these ages in the herd, crossbred or backcross populations would produce on the average relative to the Red Sindhis as follows: 1/2 Jersey, 2.09; 3/4 Jersey, 1.47; 1/4 Jersey, 1.39; 1/8 Jersey, 1.18; and 1/16 Jersey, 1.25. Thus, earlier ages at calving boost the lifetime production of the crosses and backcrosses considerably above the estimates on a single lactation basis. Figure 2 shows the estimated production of each breeding group relative to Red Sindhis. Differences in calving intervals

were not included in this calculated production because of the nonsignificance of differences in this trait. It would appear, in view of Rathore's study (8) on body sizes of the crosses that these increases in production exceed that which would be expected on only an increase in body size basis.

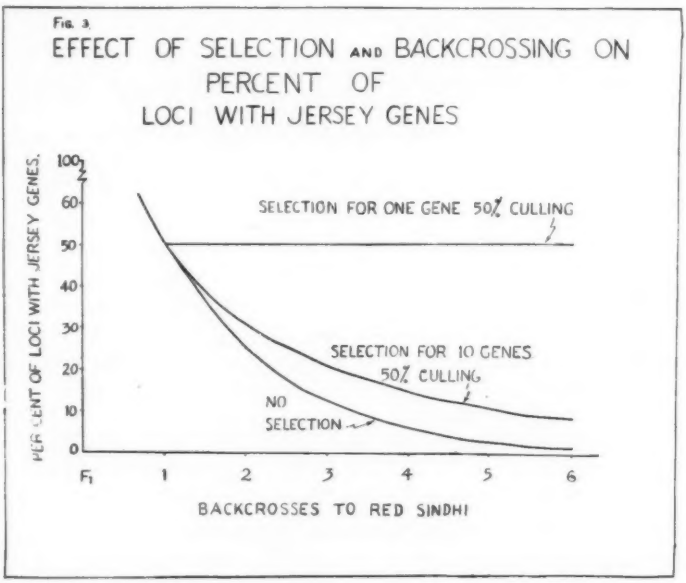
The data in this study indicated that with continued backcrossing to the Red Sindhi, little could be done to hold the production of the Jersey introduction. The original plan of crossing and then selecting from the backcrosses would be



expected to work if there were but few genes involved and if the trait were highly heritable. For example, a rather marked degree of success has been obtained in introducing the polled gene in some cattle strains in this way. However, the available evidence has indicated that the number of genes involved in explaining differences in yield in dairy cattle must be at least seven to ten pairs and very likely is due to a hundred or more pairs of genes (Lush, 7). Coupled with this, the heritability of milk or fat production is fairly low, and it would be expected that there would be many mistakes in selections. Thus, theoretically it would appear unlikely that much could be done to hold the production of the crossbred while backcrossing to the Red Sindhi. Figure 3 shows the effects of 50 per cent culling with no mistakes (100 per cent heritability) and with different numbers of genes on ability to hold desired genes from one parent breed while backcrossing to the other one of the parent breeds. In the present instance, it appeared that actually as well as theoretically the production of the backcrosses rapidly approaches the production characteristics of the Red Sindhi parent. If

the production characteristics of the Jersey × Red Sindhi cross were to be maintained some method other than that which has been used would be needed.

The points in Figure 3 were plotted by expanding binomials with one and ten gene pairs, selecting the top 50 per cent of the genotypes in the F_2 dams, and calculating for their Red Sindhi sired progeny the average number of loci-carrying Jersey genes. The process was repeated to procure estimates for subsequent backcross generations.



SUMMARY

In the herd of crossbred Jersey × Red Sindhi backcross and purebred Red Sindhis of the Allahabad Agricultural Institute, dairy performance as related to breeding has been studied. In percentage of butterfat in the milk, the crosses have shown slight but nonsignificant increases over the purebred Red Sindhis; neither have there been significant differences in the length of calving intervals or in survivability of young calves for the different groups. In comparison with purebred Red Sindhis, great differences have appeared in the advantages of the crossbreds particularly, and to a lesser degree of the backcrosses, in ages at first calving and in the production of butterfat in lactation periods of 305 days. Of the cows having first lactations, over twice the proportion of crossbred cows have remained in the herd for a period of six lactations as have purebred Red Sindhis. If total production for the number of years cows remain in the herd is computed from the data on age at first calving and production in the first lactation, then the production of a herd of F_1 Jersey × Red Sindhi would exceed by 2.09 times

that of a herd of purebred Red Sindhis. The ratios of calculated lifetime productions of populations of backcrosses to the Red Sindhi were as follows: ¾ Jersey, 1.47; ¼ Jersey, 1.39; ⅛ Jersey, 1.18; 1/16 Jersey, 1.25. These ratios were computed by weighting the number of cows of different ages by the advantage of each of the breeding groups at that age. It appeared as a result of this study that all proportions of Jersey breeding used exceeded the production of the Red Sindhi, with the amounts decreasing as relationship to either Jersey or Red Sindhi deviated from 50 per cent.

ACKNOWLEDGMENTS

Acknowledgment is given to A. T. Mosher and J. N. Warner of the Allahabad Agricultural Institute and to the Committee on the International Exchange of Persons for making this study possible.

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ESTIMATES OF GENETIC CHANGES IN AN INDIAN HERD OF RED SINDHI DAIRY CATTLE

H. H. STONAKER[1]

Department of Animal Husbandry,
Colorado A. and M. College, Fort Collins

An important aspect of the breeding program for the Red Sindhi herd of dairy cattle of the Allahabad Agricultural Institute (India) has been efforts to improve by selection the production of the purebred Red Sindhis. The crossbreeding of Jerseys with Red Sindhi and backcrossing to Red Sindhi have been described by Stonaker, Agarwala, and Sundaresan (14) in a previous paper. It was apparent from this program of backcrossing that the recent genetic change in the herd was dependent upon the rate of improvement in the bull-producing Red Sindhi herd. The number of animals in the breeding herd has been about 120 cows of milking age, with three Red Sindhi bulls and one or two other bulls used in crossbreeding. Twenty-two purebred Red Sindhi cows were the nucleus of the bull-producing herd.

Attempts to evaluate genetic changes in farm herds have been more nearly measures of the amount of difference between those saved and culled and the generation time required than measures of actual genetic changes. Because the managers of herds attempt to improve simultaneously the genetic composition and the environmental background of the herd, estimates of long-time genetic changes are largely deductive and lack the greater surety of direct experimentation. However, such analyses are of value, for they serve as guides in evaluating the components of selection pressure. Several workers have reported on various techniques for developing a satisfactory solution to the problem. Henderson (2) has refined the method of comparing successive records of cows as a guide to the probable environmental changes in the herd. He used this method in evaluating the noncontemporary progeny of dairy sires. Estimates of genetic change have been made from heritability values and knowledge of the actual selection pressure exerted. Heritability methods have been developed by Lush (4). Dickerson and Hazel (1) later showed the relationship between various degrees of culling, age of parents, and annual genetic progress. Recently, workers in the United Kingdom, applying the methods of Lush, Dickerson, and Hazel, made estimates on rate of improvement in several herds of dairy cattle. Rendel and Robertson (7) estimated that annual improvement in a closed herd of Ayrshires was 0.7 per cent of the average yield per year. Estimates that the annual improvement in pedigree herds in the United Kingdom was 0.4 per cent of the annual yield were made by Rendel, Robertson, and Alim (8). Techniques for increasing this rate of improvement were suggested by Robertson and Rendel (10). In the Fulani breed in Nigeria, South Africa, Robertson (9) estimated the genetic gain to be 2½ gal. per year, which amounted to about 1.7 per cent of annual yield.

Received for publication January 5, 1953.

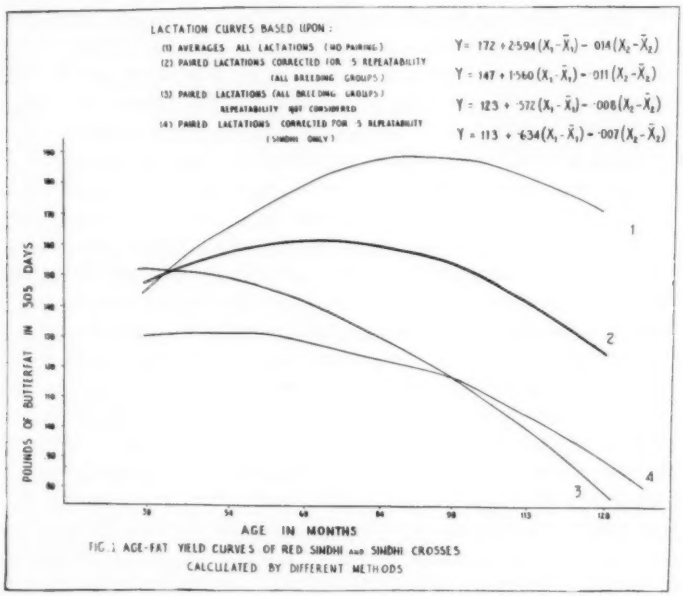
[1] Fulbright lecturer on sabbatical leave.

Adaptations of these methods have been used in this study, but, in order to make estimates of probable genetic gain, it has been necessary to evaluate the heritabilities of the various traits, the progeny tests of the sires used, the amount of culling which has taken place, the generation intervals, and the effect of age on production.

Genetic history of the Allahabad Agricultural Institute Red Sindhi herd. Red Sindhi cattle do not constitute a breed, as thought of by Western standards, for registration of these cattle is a relatively recent government-sponsored innovation. The cattle have a common origin in that they come from the Sind Province of Pakistan. In the Institute herd, according to Saxena (11), there were five purchases of foundation cows. The first thirty were purchased about 1923 on the open market in Sind; twelve were purchased in 1929, sixteen in 1932, eight in 1935, and the last twelve in 1946. This last group had no effect on the herd and was later culled in its entirety. The parentage of the foundation cows is unknown; it is said that they were selected as typical representatives of the Red Sindhi breed type. Early bull purchases included five from the Poona Agricultural College. These bulls were pedigreed in the sense that their parents and grandparents were for the most part identified. The two Poona-bred bulls, Kabir (99) and King Carr (87) were used extensively in the Institute herd and had first lactation daughters appearing in the milking herd from 1938 through 1948. In addition, some home-bred bulls have been used. No further introductions have been made which affected the herd in this study. A pedigree map of the herd, tracing to the introductions which had descendants in the herd during the period April 1949 to March 1951, was prepared. The inbreeding coefficients of the 1949-1951 calves averaged 7 per cent. Of the purchased animals, two bulls and one cow have the highest direct relationship to the 1949-1951 sample. Sind Queen (442) and Kabir were 20 per cent directly related to the 1949-1951 calf crops. King Carr was 16 per cent directly related to these calves. Sind Queen was for many years the heaviest producing Red Sindhi cow in the herd. Kabir sired 61 purebred and backcross daughters which went into the milking herd, and King Carr sired 52 daughters which had at least one lactation in the herd.

Effect of age on production. In making heritability estimates, it appeared that in view of probable great differences in environment over a period of time, the dams should be compared within the same year of production. It was impossible to do this on the basis of the dams' first lactations because of the few daughter-dam pairs that could be derived from the data on this basis. Since it is a prerequisite of the heritability estimate that the daughters are an unselected group, it was necessary to use their first lactation records. Therefore, the records of dams made in the herd in the same year were age-corrected and used. Age effects are not commonly taken into consideration in India when using the dams' records as criteria in selection. Although Sikka (12) had reported age correction terms for Indian cattle based on the widely used technique of paired lactations of the same cows, and Lush and Shrode (5) had developed correction terms for dairy cattle based on this method, it appeared that further investigation was needed in the present herd analysis.

From the Institute herd data, four different production-age curves were developed. These are shown in Figure 1. The first curve was developed by averaging the yields and ages of all cows in a given lactation. This method, as mentioned previously, included cow differences as well as age differences. The second method applied was that of paired lactations. It eliminated the effect of changes in herd population from lactation to lactation. This study yielded results differing markedly from those of previous studies made on the same basis,


LACTATION CURVES BASED UPON :
(1) AVERAGES ALL LACTATIONS (NO PAIRING)
(2) PAIRED LACTATIONS CORRECTED FOR 5 REPEATABILITY (ALL BREEDING GROUPS)
(3) PAIRED LACTATIONS (ALL BREEDING GROUPS) REPEATABILITY NOT CONSIDERED
(4) PAIRED LACTATIONS CORRECTED FOR 5 REPEATABILITY (SINDHI ONLY)
Y= 172 + 2·594 (X1 - X̄1) - 014 (X2 - X̄2)
Y = 147 + 1·960 (X1 - X̄1) - 011 (X2 - X̄2)
Y = 123 + ·572 (X1 - X̄1) - ·008 (X2 - X̄2)
Y = 113 + ·634 (X1 - X̄1) - ·007 (X2 - X̄2)
POUNDS OF BUTTERFAT IN 305 DAYS
AGE IN MONTHS
1
2
3
4
FIG. 1. AGE-FAT YIELD CURVES OF RED SINDHI AND SINDHI CROSSES CALCULATED BY DIFFERENT METHODS

as shown in curve 3 of Figure 1. The first lactation records were the highest, and the production dropped off consistently in succeeding lactations. Robertson (9) noticed this same phenomenon in the Fulani herd he studied in Nigeria, although it was not mentioned whether it was observed as the result of pairing records of individual cows. Correction terms derived from this method of paired lactations gave very high corrections for the aged cows in the herd and led to corrected records for some of these older cows which exceeded any yields which had been produced in the herd. The combination of the effects of incomplete repeatability of records and selection on first records result in the first of a pair of lactation records of a cow being an overestimate of her later producing ability. Thus, while the first of a pair of records would be subject to temporary environmental influences on selection, the second record of the pair was free from this. The first records of each pair were corrected for this incomplete repeatability by multiplying the difference between the records before and after culling by the repeatability of 0.5. (The correlation between first and second and second and

third records was 0.5 in this study.) This difference then was added to the average of all cattle before culling and used as the real producing ability of the cows which remained for a succeeding lactation. The use of this method modified the correction terms considerably and resulted in the third lactation being the highest, but with slight age correction up to the fifth lactation when production dropped rapidly. (Curve 2 in Figure 1.) For the purebred Sindhi the curve was slightly different, and, with first records adjusted for incomplete repeatability, the first lactation remained slightly the highest. The correction terms were developed on the basis of correcting all lactations to a first lactation basis. In all cases the curvilinear regressions of production on age were calculated by methods as outlined by Snedecor (13).

Selection pressure on production characteristics of cows. Annual progress which may be expected in a trait is the product of the heritability of the trait and the annual selection pressure exerted on the trait. The latter is the result of both automatic and intentional culling. In the present study it was possible to measure selection pressure by calculating the average of the first lactation records of all cattle and then comparing this with the average of the first lactation records of the animals remaining in the herd for a second lactation. The process was continued to discover, as well, how much selection pressure had been made on 305-day production after the second, third, fourth, fifth, and sixth lactations. The results indicated that there was continued selection pressure among cows for increased production through the fourth lactation. Two other traits also were examined because of their bearing on total productiveness of dairy cattle. These were age at first calving and length of time between calvings. Again the measure of culling was based upon the average age at first calving and length of time

TABLE 1

Selection of Red Sindhis for fat production, calving age, and first calving interval

	Before culling	After culling	Before culling	After culling
Lactation No.	(1)		(4)	
No. of cows	59	46	22	13
Lb. fat	134	150	115	135
Age at 1st calving *(mo.)*	40.5	40.7	36.8	37.2
1st calving interval	.....	.....	15.4	15.7
% surviving	.....	78	.....	22
Lactation No.	(2)		(5)	
No. of cows	46	30	13	9
Lb. fat	135	146	156	155
Age at 1st calving *(mo.)*	38.8	38.9	.....	.....
1st calving interval	17.6	17.3	.....	.....
% surviving	.....	51	.....	15
Lactation No.	(3)		(6)	
No. of cows	30	22	9	5
Lb. fat	145	146	122	119
Age at 1st calving *(mo.)*	37.9	37.6	.....	.....
1st calving interval	15.8	16.3	.....	.....
% surviving	.....	37	.....	8

between calvings for all cows, as compared with the average of those which remained in the herd for another lactation period. For these two latter traits, there appeared to have been no effective culling, as is recorded in Table 1.

The advantage in production of those saved was particularly large in the first, second, and fourth lactations. For ages at first calving there was little difference between the general average and those saved for subsequent lactations. Thus, it did not appear that much selection was taking place on age at first calving.

Heritabilities of various traits. It is well established in numerous studies that heritabilities of various traits show a wide range in values (Phillips, 6). As has been pointed out, for traits of equal economic importance it is more important to stress those which have the greatest heritability, for they will be the most responsive to selection. Two methods are commonly used for estimating the heritability of a trait from herd data. The regression of offspring on dam is doubled, or the intra-class correlation between paternal half sibs is quadrupled, to give an estimate. It is important that the offspring used be contemporary because of time changes in environment. Also they must represent unselected, or random samples of the offspring. In the present analysis it was necessary to use the intra-sire regression of daughters on dams in which all of the daughters were by Red Sindhi bulls, but the dams may have been crossbreds, various grades of Red Sindhi, or purebred Red Sindhis. Since the estimates in the study were of the genetic variation among the dams, they would be expected to be somewhat higher than if they had been on a purebred Red Sindhi population. Because of the limitations in number of data from the one herd it was not feasible to compare estimates based upon a population of crossbred dams versus one calculated from purebred dams. For butterfat production, the unselected first lactation records of daughters milking in the same year and by the same sire were regressed upon the age corrected records of their dams. Only the dams which had lactation records in the same year were compared, but their records were not made necessarily in the same years as their daughters' records. The method of paternal half sibs was not suitable because of the few sires used in a given year. Heritability of per cent fat in the milk was based upon the same arrangement of daughter-dam pairs as in the butterfat yield study. It also was necessary to use

TABLE 2

Heritabilities of traits in the population of crossbred, backcross, and purebred Red Sindhi dairy cattle

Trait	Degrees of freedom	Heritability	Trait	Degrees of freedom	Heritability
Total butterfat in 305 days[a]	92	0.25	6-mo. weight	20	— 0.05
			2-yr. weight	21	0.18
% butterfat	82	0.09	Mature height	18	0.78
Birth weight	37	— 0.09			
Birth height	10	0.16	Age at first calving[b]	90	0.39
			Calving interval[c]	41	0.88

[a, b, c] Standard errors: 0.28, 0.16, 0.46, respectively.

the same arrangement for estimating heritabilities of such things as weight, height, age at first calving, and calving interval. For these latter studies this method of pairing left much to be desired, for although the daughters generally were under the same herd environment, dams within the group may have been under different environments when they were of an age to have their weights and measurements taken. The error terms of the heritability estimates were calculated for three traits which had the greatest number of degrees of freedom. These were so large as to cause almost any heritability value to be within the fiducial limits of the ones obtained. The heritability estimates are presented in Table 2. In spite of the large sampling error, these estimates are some evidence on selection opportunities. Heritability of total butterfat production was slightly higher than the estimates which have been reported for various breeds in the United States (Tyler and Hyatt, 15); it was lower than that reported in the study of the Fulani breed (Robertson, 9). In view of other studies (Tyler and Hyatt, 15) fat percentage heritability was particularly low. Mature height corresponded with that made in American studies. Ages at first calving and calving intervals were highly heritable.

Incidental to the heritability studies, the phenotypic correlations between traits were investigated. Sire and year of first lactation were held constant in computing these pooled correlations. They are shown in Table 3. The higher

TABLE 3

Intra-sire, intra-first lactation year correlations between various traits of Red Sindhi and backcross females

Variables	Correlations	Variables	Correlations
Lb. fat . butterfat %	0.097	Butterfat % . mature ht.	0.006
Lb. fat . birth wt.	0.085	Birth wt. . 6-mo. wt.	0.316[a]
Lb. fat . 6-mo. wt.	0.169	Birth wt. . mature wt.	0.261
Lb. fat . mature wt.	0.140	Birth wt. . mature ht.	0.542[b]
Lb. fat . mature ht.	— 0.060	6 mo. wt. . mature wt.	0.327
Butterfat % . birth wt.	0.002	6 mo. wt. . mature ht.	0.237
Butterfat % . 6-mo. wt.	— 0.036	Mature wt. . mature ht.	0.676[b]
Butterfat % . mature wt.	0.026	Lb. fat in succeeding lactations	0.500[b]

[a] Significant at 0.05
[b] Significant at 0.01

correlations were those between weights and measurements of the same animals. Rather striking, however, were the low correlations between size and 305-day production.

Progeny tests of the sires. Five of the sires at the Institute had been used sufficiently to evaluate their progeny tests. Of the five, Kabir sired the most daughters in the herd over an 11-year period, and he was used as a check for ranking the merit of the other bulls. Kabir's daughters in the control group had first lactations in the same years as those in the compared groups. Thus the composition of the controls differed from time to time. The differences between the daughters' averages were corrected for the differences between the mates' averages. The ranking of the sires is shown in Table 4.

TABLE 4

Sire evaluations and the number of their Red Sindhi daughters

Sire comparisons	Av. butterfat differences in lb. between daughters of compared sires and between mates of compared sires			Rank of sires	Lb. B. F. sire 87 was exceeded	No. of Red Sindhi daughters
	Daughters (D)	Mates (M)	2D-M			
99-97	-27.3	30.0	-84.6	448	124	5
99-448	-46.0	-10.0	-82.0	617	123	14
99-617	- 3.0	75.2	-81.2	99	42	23
99-87	4.5	-33.5	42.5	87	0	14

The assumption was made that the average merit of these bulls should be a reasonable base from which to estimate the degree to which merit of sire determined the number of his daughters brought into the herd. Weighting the amount which each sire exceeded King Carr (the lowest ranking sire) by the number of his daughters gave an estimate of the total genetic change due to the sires used.

It was not known precisely how these sires compared with those in other herds. Indications were gained by comparing on an intra-year basis the first lactation records on Institute-bred Red Sindhis with the contemporary but not first lactation records of purchased Red Sindhis. Since the birthdates of the purchased Sindhis were not known, their records were not corrected. This introduced a bias in favor of the Institute Red Sindhis. The purchased cows were undoubtedly subjected to some selection in the herd and at the time of purchase, although lactation records were not available at the time of purchase. Thus, the comparison of purchased versus home-bred animals is comparing a selected group with an unselected group but with some disadvantage to the selected group because of age. The weighted difference was 7.45 lb. of butterfat advantage per lactation for the purchased Sindhis over a 9-year period, in which 62 lactations of purchased cows were compared with 49 first lactation Institute-bred cows.

Estimated annual genetic change. Estimates of annual genetic gain were adaptations of the methods of Dickerson and Hazel (1). By their procedures, annual genetic gain =

$$\frac{\text{genetic superiority of dams} + \text{genetic superiority of sires}}{\text{av. age of dams} + \text{av. age of sires when progeny were born.}}$$

In this study the estimated total genetic gain in 16 years resulting from larger numbers of progeny from the better sires was 1.53 lb. of butterfat. This amounted to 0.1 lb. of butterfat per year or 0.7 lb. per generation of 7.29 years (the average age of the sires when their daughters were born). The estimate of genetic gain from the dams' side was obtained by calculating the phenotypic reach made as a result of the survival in the herd of the higher producing Sindhi cows. These values are shown in Table 1. This sum of the phenotypic reach was multiplied by the heritability estimate of 0.25 to provide an estimate of the average genetic superiority of the dams. The total estimated annual genetic gain in butterfat was then as follows:

$\{0.25\,[(0.78)\,(16) + (0.51)\,(11) + (0.37)\,(1) + (0.22)\,(20) + (0.15)\,(-1) + (0.08\,(-3)\,] + 0.7\,\}/(6.00 + 7.29) = 0.48$ (lb. per year).

As a percentage of average first lactation butterfat yield this amounted to about 0.3 per cent per year, an estimate which was a bit lower than that of Rendel, Robertson, and Alim (8) for probable average annual genetic increase in production of United Kingdom pedigreed dairy herds.

DISCUSSION

The estimates of progress in increasing production in this study were not discouragingly low in view of the small size of the Red Sindhi herd and the long generation intervals, and considering estimates of change in other dairy herds. However, recent information on techniques for increasing change would indicate, in view of this study and the preceding one on the performance of crosses and backcrosses, that there may be opportunities to move ahead faster in the future.

If the present program of backcrossing to Red Sindhi continues there will be, as pointed out in the previous study, an almost certain continued drop in production per cow. However, an advantage will result from increased selection opportunities in a larger registered herd, for cattle with 31/32 Red Sindhi breeding may be registered. If the Red Sindhi cow herd grew from 22 to 122, there would be an opportunity for doubling the selection pressure on the dams of bulls. This would require that the herd be interbred in order to avoid the possibility of halving the amount which the herd pulls ahead of the breed average. Interbreeding in a herd of four sires and 120 cows would not need to cause the inbreeding to rise at a very rapid rate.

Efficiency of selection also could be increased by using 305-day lactation periods rather than average daily yield during the entire lactation or yield in the total lactation, for the latter methods tend to favor unusually short or long lactations. Weighting of information on 305-day records of the individual, or relatives, or both, as developed in Legates' (3) selection index, also should increase the effectiveness of selection of cows, young bulls, and heifers.

As pointed out by Dickerson and Hazel (1), progress is also a function of the generation interval. The greatest opportunity for shortening this in the present herd would be in the use of younger bulls. The goal might well be to begin mating the bulls as soon as possible and to limit their use to the production of 25 to 30 calves. The bulls might then be used on village cattle until their daughters' records were known. Proved bulls could be returned for further use where the genetic advantage of bringing them back into the herd is not cancelled by the corresponding increase in generation interval. If the effects of increased size of the herd and decreased age of bulls could be achieved it would appear that increases due to selection might be doubled.

A properly weighted index including calving interval and age at first calving might produce a considerable increase in selection pressure for production per cow during a given period of time over selecting for production per lactation

alone. Such a study would require more information on the economic importance of, and the genetic correlations between, these traits.

Lastly, anything which could be done economically to decrease the high death losses of heifer calves reported in the previous study would increase selection differentials through enlarging opportunities for culling on first lactations or in early disposal of those with inferior parents.

These goals for improving selection would entail considerable management effort and in some cases additional cost. It does seem likely that an achievement of one per cent increase in average per year could be attained, as indicated by Rendel and Robertson (7) as being the probable maximum in a herd of about the same size as this but using United Kingdom heritabilities and replacement rates. If this could be done, and if the theoretical assumption is correct that selection effects are cumulative and of a compound interest nature, then in 70 years one might have a purebred Red Sindhi herd producing as well as the first generation Jersey $\times$ Red Sindhi crossbreds produced in this herd.

SUMMARY

In the Red Sindhi dairy cattle herd of the Allahabad Agricultural Institute, which has been almost completely closed to outside breeding since 1934, the inbreeding of calves in 1949-1951 was about 7 per cent. Relationships were highest to Sind Queen, the highest producer of the foundation cows. She was the dam of two herd sires. Two herd sires purchased in 1935 also had a strong genetic influence on calves born in 1949-1951. These sires, King Carr and Kabir, had progeny born in the herd over an 11-year period.

The purebred Red Sindhi herd made up about one-sixth of the total milking herd and was the source of bulls for the entire herd. The estimated annual increase resulting from the better bulls siring more daughters and from the culling of lower-producing cows resulted in estimated increases of 0.5 lb. of butterfat per year or about 0.3 per cent of the average production per year. Heritabilities of calving interval, age at first calving, and measurements were above 30 per cent. Lower heritabilities were found for 305-day butterfat production, per cent butterfat in milk, and body weights.

Age corrections for purebred Red Sindhis developed by correcting paired lactations for incomplete repeatability indicated that the first lactation may be the most productive. For all cattle, including crossbreds and backcrosses, the third lactation was the highest, with but very little correction until the cattle reached the fifth lactation.

ACKNOWLEDGMENTS

Acknowledgment is given to A. T. Mosher and J. N. Warner of the Allahabad Agricultural Institute and to the Committee on the International Exchange of Persons for their assistance.

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THE EXTRACELLULAR LIPASES OF SOME GRAM-NEGATIVE NON-SPOREFORMING ROD-SHAPED BACTERIA [1]

S. A. NASHIF AND F. E. NELSON
Dairy Industry Section, Iowa Agricultural Experiment Station, Ames

The characteristics of the lipase of *Pseudomonas fluorescens* have been studied by Söhngen (10), Tammisto (12), Lubert *et al.* (7), and others. Detailed studies have been made also on the lipases of *Bacillus prodigiosum (Serratia marcescens)* by Tammisto (12) and of *Pseudomonas fragi* by Nashif and Nelson (8). However, the work on the lipases of related organisms is fragmentary.

Thermostability of the lipases of *Ps. fluorescens* (11, 12) and *Ps. fragi* (8, 10) has been reported. Pasteurization temperatures commonly used for cream for butter making are ineffective for inactivation of the lipase of the latter organism (10). These results suggested the possible existence of other heat-resistant lipases produced by related bacteria. Further knowledge of additional characteristics of the lipases of certain organisms sometimes found in dairy products seemed desirable.

EXPERIMENTAL METHODS

The methods for measurement of lipase activity, studies on the pH of the test substrate, treatment of cultures, and heat inactivation were reported previously (8). An exception in the present study was the use of water baths at 80 to 85 and 71.6° C. to bring the lipase preparations up to the temperatures of 71.6 and 61.6° C., respectively, in about 1 minute instead of 3 to 4 minutes when the tubes were heated in baths set at the temperatures used for holding. Also, cream was neutralized to pH 6.5-6.6 before heating at 71.6° C. for 30 minutes.

The cultures used in this study and the sources from which they were obtained are listed in Table 1. The cultures were from a group of 23 that showed lipolysis on coconut oil agar plates by the nile blue sulfate technique of Collins and Hammer (3). These organisms were selected for further study because of their ability to produce measurable lipase activities in phosphate-buffered peptone broth at 21° C. within 3 days. Only extracellular lipases were considered, since previous studies with *Ps. fragi* had established that little enzyme activity was associated with the cells removed by centrifugation.

The cultures were checked as to their taxonomic position by their bio-chemical, physiological, and morphological characteristics. In the differentiation of members of this group, the type of flagellation is important and this was determined by flagella staining (2) and by electron microscopy.[2] Culture P-63 was labeled as *Pseudomonas* sp. on receipt, but was found to resemble *Ps. fluorescens*. Cul-

Received for publication January 10, 1953.

[1] Journal paper No. J-2149 of the Iowa Agricultural Experiment Station, Ames. Project No. 1050.

[2] The assistance of P. H. Carr of the Physics Department is acknowledged.

ture P-600 resembled *Ps. fluorescens* but differed in having lophotrichous flagella, and culture P-83 could not be identified by any of the descriptions of members of the genus *Pseudomonas* given in Bergey's Manual (1). *Pseudomonas viscosa* E17 did not show viscid growth on agar and *Achromobacter lipolyticum* was found to be Gram-negative but otherwise to possess the characteristics ascribed to the species by Bergey (1).

TABLE 1
Source of cultures

Culture No.	Name	Source
P-63	*Pseudomonas sp.*	J. J. Jezeski
P-83	*Pseudomonas sp.*	Univ. of Minn.
P-90	*Flavobacterium sp.*	
P-600	Unknown	
E-1	*Ps. aeruginosa*	J. E. Edmondson
EJ5	*Ps. synxantha*	Univ. of Mo.
E17	*Ps. viscosa*	
.....	*Achr. lipolyticum*	W. O. Ne'son, Univ. of Ill.
ISC 2F4	*Ps. aeruginosa*	R. N. Doetsch
AN69	Unknown	Univ. of Md.
.....	*Alc. viscosus*	M. P. Baker
.....	*Ser. marcescens*	Iowa State College
0-1	*Ps. fragi*	Iowa State College

RESULTS

Many of the cultures tested showed good lipolysis on agar plates containing coconut oil emulsion and nile blue sulfate, but some of these cultures failed to produce measurable extracellular lipase in 3 days on phosphate-buffered peptone broth or nutrient broth at either 21 or 32° C. Lipase production usually was depressed when nutrient broth instead of the peptone broth was used. With the exception of *Achr. lipolyticum* and *Ps. fragi*, the addition of caprylic acid in 0.05 per cent concentration in peptone broth (9) caused no appreciable increase in lipase production by the organisms tested.

One of the better known characteristics of lipases that varies from one enzyme source to another is the optimum pH for activity. Figure 1 shows the effect of pH upon activity of the various lipases tried. Final pH values for the medium were plotted because there were appreciable drifts in pH to the acid side during the reaction period of 24 hours at 36° C. The buffering capacity of the phosphate buffer decreased with the increase in pH, with an average reduction of 0.2 pH units from the initial pH values. Boric acid buffer allowed greater shifts in pH; with an initial reaction of pH 8.0 the shift amounted to 0.2-0.7 pH units, but the shift decreased at the higher pH levels.

Lipases of all of the cultures had their optima at slightly above pH 7.0. *Ps. aeruginosa* E1 and *Alc. viscosus* produced lipases that remained fairly active above pH 9.5. Most of the lipases hydrolyzed coconut oil over a wide range of

pH. The lipase activity curves obtained with test substrates buffered with boric acid were on a lower level than those with which a phosphate buffer was used, hence the discontinuity of the curves. This probably is due to the inhibitory action of the borate ion upon lipase activity. The emulsion used for enzyme substratum was buffered at pH 7.2 ± 0.05 in subsequent studies.

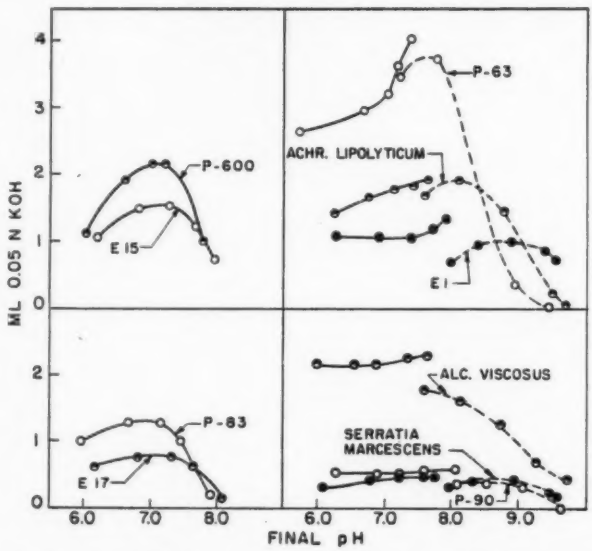

FIG. 1. The influence of pH on the activities of the various bacterial lipases. (Plotted as ml. 0.05 *N* KOH to titrate the free fatty acids extracted from 10 ml. of the 40 ml. of enzyme-substrate mixture incubated 24 hr. at 37° C.)

The data presented in Table 2 on the effect of temperature of culture incubation upon lipase production by the various organisms were obtained in two trials. Based upon the enzyme activity in the centrifuged broth, *Achr. lipolyticum* showed the highest lipase activity after incubation at 32° C., P-63 and P-600 at 15° C., and the remaining organisms at 21° C. In many instances, the higher lipolytic activities could be associated with greater growth, as indicated by plate counts. Calculating enzyme activity per billion count, *Ser. marcescens* joins *Achr. lipolyticum* in having the optimum at 32° C. On this basis, most of the other organisms have optima at 21° C., *Alc. viscosus* and *Pseudomonas* sp. P-83 being the exceptions with optima at 15° C. The results also show the marked differences in the amounts of extracellular lipolytic enzyme activity produced by the various organisms.

Heat resistance of lipases, particularly to temperatures and exposures that have been thought in the dairy industry to inactivate defect-producing enzymes and microorganisms, is of vital concern. As shown in Table 3, six organisms

produced lipases that were not completely inactivated by heating in phosphate-buffered peptone broth at 71.6° C. for 30 minutes. In addition to *Ps. fragi* 0-1, these organisms included *Achr. lipolyticum*, *Ps. synxantha* E15, *Ps. fluorescens* P-63, *Pseudomonas* sp. P-600, and culture AN69. *Alc. viscosus*, *Ps. aeruginosa* E1, and *Ps. aeruginosa* ISC 2F4 produced lipases that remained appreciably active in broth heated at 61.6° C. for 30 minutes, but not at 71.6° C. for 30 minutes. The lipases of the remaining cultures were rather heat sensitive.

Greater destruction of lipase activity at 61.6° C. than at 71.6° C. was observed in the case of *Achr. lipolyticum*, *Ps. fluorescens* P-63, *Ps. fragi* 0-1, and

TABLE 2

Effect of temperature of incubation upon extracellular lipase production in peptone broth by some organisms (cultures incubated 3 days)

Culture	Temp. (° C.)	Final [a] pH	Count / ml.[b] (millions)	Ml. lipase prepn. per 40 ml. substrate	Net [c] activity	Calculated activity / billion count
Achr. lipolyticum	15	7.83	2,100	2.0	0.54	0.13
	21	8.08	1.400	2.0	1.33	0.48
	32	7.92	420	2.0	3.00	3.57
Ps. aeruginosa E1	15	7.41	320	1.0	0.03	0.09
	21	7.70	1,500	1.0	2.32	1.54
	32	7.83	1,800	1.0	0.91	0.50
Flavobacterium sp. P-60	15	7.24	490	2.0	0.60	0.61
	21	7.33	500	2.0	1.48	1.48
	32	7.27	370	2.0	0.21	0.29
Ps. synxantha E15	15	7.54	1,800	1.0	0.79	0.43
	21	7.61	2,200	1.0	1.29	0.59
	32	7.78	830	1.0	0.32	0.39
Ps. viscosa E17	15	7.44	160	2.0	0.54	1.69
	21	7.46	290	2.0	1.15	1.99
	32	7.42	70	2.0	0.00	0.00
Pseudomonas sp. P-83	15	7.52	1,000	1.0	1.31	1.31
	21	7.62	2,100	1.0	1.75	0.83
	32	7.66	420	1.0	0.13	0.31
Alc. viscosus	15	7.31	50	1.0	0.50	10.0
	21	7.33	900	1.0	2.67	2.97
	32	7.37	170	1.0	0.34	2.00
Ser. marcescens	15	7.46	1,400	1.0	0.17	0.12
	21	7.76	3,300	1.0	0.68	0.21
	32	7.74	900	1.0	0.49	0.54
Ps. fluorescens P-63	15	7.62	3,300	0.5	4.30	2.60
	21	7.79	1,500	0.5	3.47	4.62
	32	7.83	20	0.5	0.02	2.00
Pseudomonas sp. P-600	15	7.77	4,700	0.5	3.26	1.38
	21	7.67	1,700	0.5	2.06	2.42
	32	7.78	690	0.5	0.01	0.04

[a] Initial pH = 7.13

[b] Prior to removal of cells by centrifugation

[c] Net activity = ml. of 0.05 *N* KOH to titrate the acids extracted from 10 ml. of the enzyme-substrate mixture, with a correction for a blank (0.27 ml. in this series). Incubated for 24 hr. at 37° C. before extraction (8).

AN69. These results were confirmed several times. The possibility exists that some lipase inactivating agents are destroyed comparatively more rapidly at the higher temperature, thus allowing greater activity of the residual lipase. No tests were made for proteolytic activity of the enzyme preparations used.

TABLE 3

Effect of heat [a] *on the activity of extracellular lipases produced by some Gram-negative, non-sporeforming, rod-shaped bacteria*

Culture	Net lipase activity [b] (corrected for blank)				
	Unheated	After heating at 61.6° C. for		After heating at 71.6° C. for	
		0 min.	30 min.	0 min.	30 min.
Achr. lipolyticum	2.97	1.65	0.34	1.71	1.57
E15	0.75	0.44	0.38	0.44	0.39
P-63	3.06	1.05	0.26	1.34	1.06
P-600	1.40	1.28	0.86	1.40	0.85
0-1	1.00	0.60	0.26	0.52	0.33
AN69	0.70	0.26	0.06	0.36	0.22
Alc. viscosus	1.11	1.01	0.44	0.90	0.00
E1	0.45	0.41	0.28	0.29	0.01
ISC 2F4	1.09	1.07	0.94	0.44	0.00
E17	0.28	0.07	0.00	0.00	0.00
P-83	0.33	0.05	0.01	0.02	0.00
P-90	0.45	0.44	0.09	0.29	0.00
Ser. marcescens	0.20	0.04	0.00	0.04	0.00

[a] Heated in broth adjusted to pH 7.0.
[b] See footnote "c" of Table 2.

Data on the course of heat inactivation of the lipases of some cultures at 71.6 and 99° C. are presented in Figure 2. The shapes of the curves indicate that the relationship between heat inactivation and time is logarithmic. The lipase

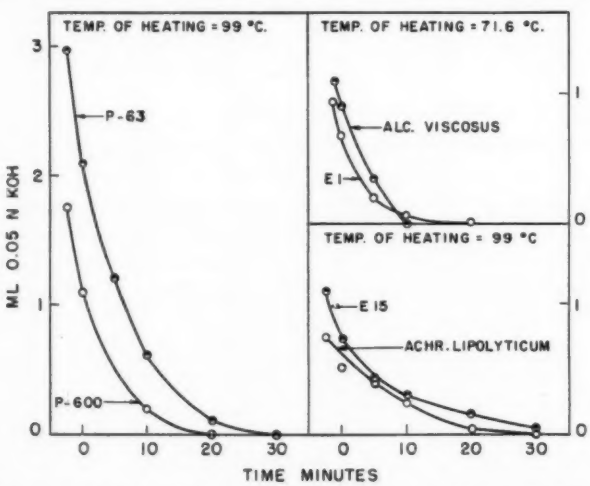

FIG. 2. The effect of heating for various periods of time on the inactivation of the various bacterial lipases.

of *Ps. fluorescens* P-63 was inactivated rapidly during the first 10 minutes but was destroyed more slowly up to 30 minutes at 99° C. The curve for the lipase of *Pseudomonas* P-600 shows the same trend, although the initial activity level was somewhat lower. These results confirm the previous findings of Tammisto (12) and Söhngen (11) relative to the unusual heat stability of this enzyme. The lipases of *Achr. lipolyticum* and *Ps. synxantha* E15 showed considerable resistance at 99° C. The lipases of *Alc. viscosus* and *Ps. aeruginosa* E1 were inactivated rapidly at 71.6° C.

TABLE 4

Lipase production in cream by some organisms and effect of pasteurization at 71.6° C. for 30 min. on lipase activity (1 ml. cream per 40 ml. test substrate)

Culture	Final [a] pH	Count / ml. *(millions)*	Net activity [b]	
			Before past.	After past.
Achr. lipolyticum	5.42	1,100	0.16	0.09
E1	6.41	1,200	0.07	0.06
E15	6.29	1,200	0.21	0.07
E17	6.13	390	0.19	0.03
P-63	6.32	820	0.79	0.32
P-83	6.07	1,200	0.42	0.04
P-90	6.33	3,100	0.21	0.20
P-600	6.13	1,600	5.49	2.14

[a] Initial pH of cream = 6.36
[b] Blank deducted = 0.31; see footnote "c" of Table 2.

The ability of these organisms to produce lipase in cream within 3 days at 21° C. was not great, with the exception of culture P-600, as is shown in Table 4. In three trials, the latter culture showed a remarkable ability to produce lipase in cream, and *Ps. fluorescens* P-63 also produced appreciable lipase. Lipase production by *Achr. lipolyticum* probably was depressed because of the considerable reduction in pH during the incubation period. The lipases of cultures P-63 and P-600 both showed appreciable activity after heating in cream at 71.6° C. for 30 minutes. The lipase of *Flavobacterium* sp. P-90 was almost unaffected by the pasteurization procedure, although the enzyme was inactivated easily in peptone broth (Table 3). This unusual resistance may be due to some protective action of the cream or to the large number of cells present in the cream after the incubation period.

DISCUSSION

A discrepancy between lipolysis on agar plates containing coconut oil emulsion and extracellular lipase production in peptone or nutrient broth was noticed. The adaptive character of the lipases of certain microorganisms, as brought out by the findings of Goodman (5) and Cutchins *et al.* (4), may partly explain the above observation. However, the high production of lipase in peptone broth in the absence of any lipid material stands as an obstacle to the acceptance of such an explanation, as does the lower production in cream than in peptone broth. The earlier studies on *Ps. fragi* (9) definitely demonstrated the nonadaptive character of lipase by that organism. However, the method of choosing

the organisms in the present study would tend to eliminate those in which the lipase was truly adaptive. The cultures which failed to produce lipase detectable in the centrifuged broth may have owed their lipolytic activity on agar plates to an intracellular enzyme. This possibility was not checked. Other factors, such as oxygen relationships, might be operative.

Temperature of growth has an important effect on the extent of lipase production by microorganisms. This was shown previously to be true in the case of *Ps. fragi* (9). The ability of various lipolytic *Pseudomonas* species to produce the greatest amount of lipase at 21° C. or below is of practical significance, since these organisms may be detrimental to the quality of dairy products held at these lower temperatures to reduce microbial deterioration. The data on enzyme activity per billion count demonstrate that temperature of incubation affects not only the number of countable cells but also the enzyme production per countable cell. One must not overlook the fact that, as demonstrated for *Ps. fragi* (9), a shorter incubation period might have been more favorable to the higher temperatures for some of these organisms; not only do the organisms grow more rapidly at the higher temperatures, but also the inactivation of the enzyme proceeds at a more rapid rate. At 32° C. enzyme may be produced early but inactivated rapidly.

In the absence of fermentable carbohydrate material, the organisms studied will shift the pH of broths to the alkaline side of neutrality. Likewise the pH optima of the lipases of these organisms occur at or slightly above neutrality. Development of acidity in the product, either earlier or concurrently, would reduce lipase activity considerably. *Achr. lipolyticum* and several species of *Pseudomonas* are capable of elaborating lipases that are not completely inactivated by the pasteurization temperatures and times commonly used by the dairy industry. In most cases, the lipases withstood the heat treatments in cream better than in peptone broth, presumably because of the protective action of the cream constituents. A number of these organisms were able to produce appreciable amounts of lipase in cream. These enzymes could have deleterious effects on the product itself or the butter made from it.

Although these studies did not include all the possible organism types that may produce thermostable lipases, they serve to emphasize the importance of preventing the contamination of dairy products, especially cream, with Gram-negative lipolytic organisms so that the quality of these products may not be jeopardized by lipolytic activity subsequent to pasteurization. Not only *Pseudomonas* species but also other lipolytic bacteria may be important from this standpoint.

Since the presence or absence of lipolytic activity usually is not considered a taxonomically important characteristic of an organism and some species are known to encompass both lipolytic and nonlipolytic strains (6), there might be considerable question as to whether any importance should be attached to the characteristics of the lipase if it is present. Whether the individual cultures examined were representative of their species as far as characteristics of the

lipase produced are concerned is not known, since only examination of a much larger number of strains could establish this point.

Only in the genus *Pseudomonas* was the number of species examined such as to permit even tentative conclusions as to the possible homogenity of a genus with respect to the characteristics of the extracellular lipases produced by the various species. Most of the species of this genus that were studied produced lipase in greatest amount after incubation at 21° C. or below. The species tested all produced lipases exhibiting maximum activity at pH 7.2, or slightly above. Two cultures produced lipases which failed to retain any activity after being heated to 61.6° C. for 30 minutes in peptone broth, but the lipases of three cultures retained some activity for 10 minutes or more at 99° C., and the lipase of *Ps. fragi* is known to retain some activity after such heat treatment (8). Thus, it is evident that the lipases produced by the different species of the genus *Pseudomonas* are not homogeneous, although a number of them agreed in some of their important characteristics.

SUMMARY AND CONCLUSION

The extracellular lipases of a number of Gram-negative non-sporeforming rod-shaped bacteria were characterized in a preliminary way.

Some of the organisms that showed lipolysis on agar plates containing emulsified coconut oil did not produce detectable lipase in peptone or nutrient broths at 21° C. All of the cultures produced lipases most active at pH 7.0 or slightly above.

The optimum temperature for lipase production by the species of the genus *Pseudomonas* studied was 21° C. or below, whereas the optimum for the one culture of *Achr. lipolyticum* was 32° C.

Out of twelve cultures studied, six produced lipases that remained appreciably active in peptone broth after heating to 71.6° C. for 30 minutes.

Culture P-600, which resembles *Ps. fluorescens*, produced considerable lipase in cream in 3 days at 21° C. and *Ps. fluorescens* P-63 produced appreciable lipase, whereas the rest of the cultures were poor producers of lipase. The lipases of the two cultures remained at a high level of activity subsequent to pasteurization at 71.6° C. for 30 minutes, indicating they could cause deterioration during holding of the cream or butter made therefrom. Lower levels of post-pasteurization lipase activity were found with several other cultures.

Nonhomogeneity of the lipases produced by members of the genus *Pseudomonas* was demonstrated.

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CONTAMINATION OF MILKING MACHINE TEAT-CUP LINERS AS AFFECTED BY INCONSPICUOUS DETERIORATION OF THE INTERIOR SURFACES [1, 2]

T. J. CLAYDON
Kansas Agricultural Experiment Station, Manhattan

In milking machine sanitation, the physical condition of the rubber parts pertaining to freedom from obvious dirt, milkstone, softening, swelling, and cracking is recognized as being important in obtaining satisfactory bacteriological results. Some reports (1, 2, 3) suggest that other inherent characteristics of rubber also may be significant, and Jensen and Bortree (5) consider that bacteria may be harbored in pores of rubber teat-cup liners. Some of the discrepancies in results of investigations on milking machine sanitation, together with the consequent variations in procedures recommended for producers, well might arise from some factors that, previously, have received little consideration.

During preliminary studies at this station on a washing procedure for milking machine teat-cup clusters, inconsistent bacteriological results were obtained. These inconsistencies prevailed in spite of careful selection of rubber parts of apparently similar and satisfactory physical condition. The results obtained seemed to implicate the interior surfaces of the liners as a cause of the variations. Determination of the causes of the discrepancies led to the following investigation into the less obvious characteristics of the interior surfaces of used teat-cup liners and their effect on contamination and sanitation. The chief objective was to determine whether inconspicuous breakdown of the rubber was of importance in the susceptibility of liners to contamination.

METHODS

Teat-cup liners were obtained, in sets of four, from the College dairy farm and from four other grade-A milk producers. Efforts were made to procure liners currently in service and that were considered to be in satisfactory physical condition with no obvious surface breakdown of the rubber. During the investigation some obviously unsatisfactory liners also were obtained. The liners included several types and represented various previous sanitizing treatments and varying periods of service. In the laboratory the liners were inspected macroscopically for general physical condition. They then were turned inside out, and the interior surfaces were examined carefully with the aid of a stereoscopic, wide-field microscope at 85 × magnification. After the initial examination, the liners were cleaned thoroughly by soaking in organic acid solution [3]

Received for publication January 13, 1953.

[1] Contribution No. 220, Department of Dairy Husbandry, Kansas Agricultural Experiment Station.

[2] This study was supported by funds provided under the Research and Marketing Act of 1946.

[3] Commercial preparation used as directed for removal of milkstone.

followed by boiling in 2 per cent lye solution for 15 minutes. After being washed and dried, the liners were examined as before.

Bacteriological studies. In order to determine the importance of surface condition of the rubber liners in sanitation problems, bacteriological studies were made on the used liners in comparison with new liners of the same type having no surface imperfections. At the start of each comparison, the cleaned used liners and the new liners were steam sterilized. They then were contaminated by either experimental laboratory procedures or by usage under practical operating conditions.

Experimental contamination was accomplished by the artificial "milking" method previously reported (4). It seemed probable that the flexing of the rubber that occurs in this procedure would contribute to the contamination of teat-cup liners, especially where surface imperfections or breakdown existed. In each comparison a new and a used set of liners were contaminated from the same lot of incubated raw milk within a 10-minute period. Three liters of milk were used for a "milking" with each set of liners. After the contamination procedure, liners were removed from the claw, rinsed briefly under a water faucet to flush out free milk droplets, drained, and then examined bacteriologically. No other sanitizing procedures were used in this phase of the study since the object was to determine the initial differences in bacterial counts caused by surface condition of the rubber.

In the trials conducted under practical operating conditions, used liners that appeared satisfactory were placed on one milker unit and compared with new liners on a second unit of the same type under routine conditions at the College dairy barn. During a 2-week period, the teat-cup clusters were brought to the laboratory periodically for bacteriological examination of the liners. The clusters were obtained at the barn soon after milking and before being washed. In the laboratory, each was flush-washed by drawing through it a mild detergent solution at 110° F. to remove free milk and gross dirt. No other sanitizing treatments were applied. The liners then were removed from the claw, drained, and examined bacteriologically.

Bacteriological examinations were made by the pulsation-vacuum procedure previously described (4). The suction and squeezing action achieved would seem to be important in removing bacteria from liner surfaces, particularly when surface breakdown of the liner is involved. Thirty-five ml. portions of water rinse were used per liner, and bacterial counts were made by Standard Plate Methods with tryptone glucose extract agar. Examinations were made on liners individually, so that any relation between physical condition of a liner and its bacterial count might be determined.

RESULTS

General physical condition of liners. During the investigation, 13 sets of used liners were obtained. They had been in service for periods ranging from less than 1 month to approximately 5 months. Eight sets were in good or fairly good

physical condition as indicated by macroscopic examination before the acid and lye cleaning treatment. Their condition ranged from no apparent wear to a slight checking of the interior surface visible on close scrutiny. Sometimes light

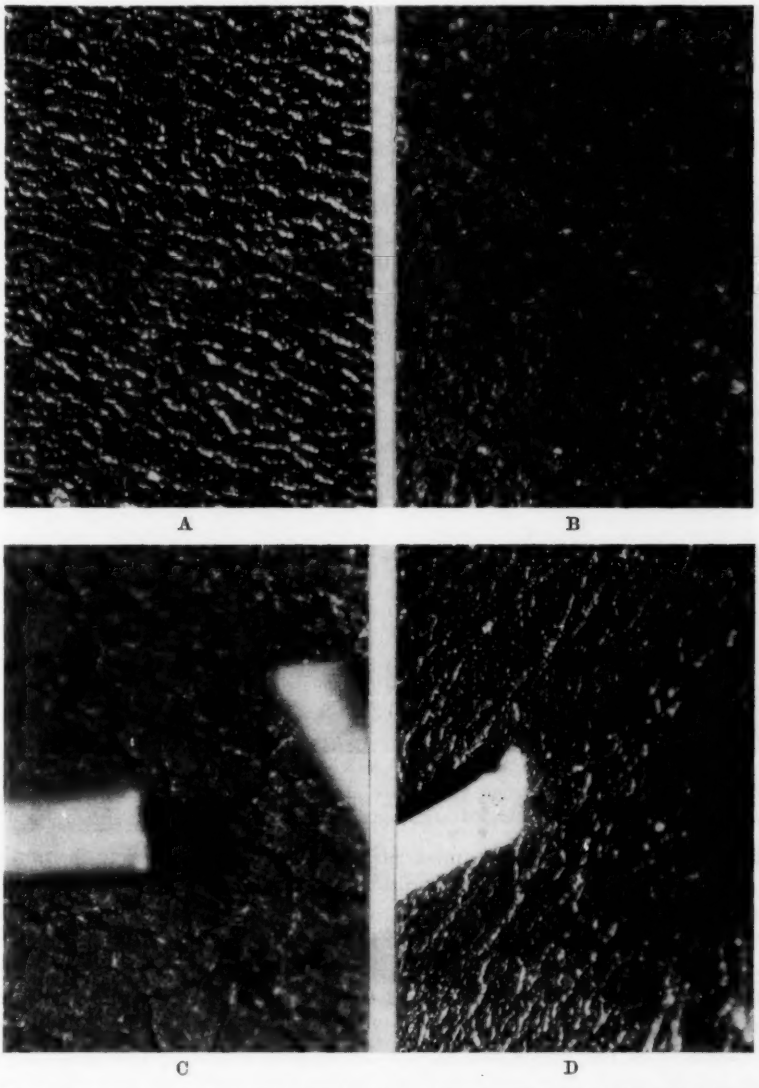
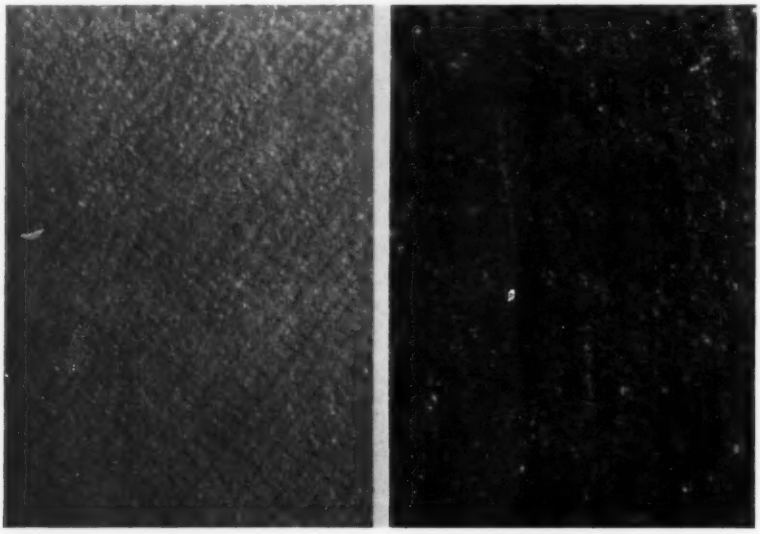


FIG. 1. Examples of microscopic appearance of interior surfaces of the used liners. In photomicrographs C and D are shown bristles from a teat-cup brush as indication of the size of the cracks. Cracks such as the large one in C are sometimes visible macroscopically as light checking. Magnification approximately 60×.

film deposits were present. These liners were smooth on the inside surface and showed little swelling or stretching. Softening or "tackiness" of the rubber rarely was evident, and conditions characteristic of fat absorption were not obvious. Five sets of liners were in less satisfactory condition. Some of these liners showed roughness and obvious checking on the inside surface occasionally combined with a heavy film deposit. Others were stretched or swollen or slightly "tacky" with indications of some fat absorption. The latter condition was infrequent in the liners obtained in this study.

The cleaning treatment generally improved the apparent condition of the liners. Among the better liners many appeared almost like new except for a slight dullness on the inner surface. Where film deposits had been removed, the



A B

FIG. 2. Examples of microscopic appearance of interior surfaces of new liners. Some of the whitish particles are dust. Magnification approximately 60×.

rubber surface was smooth and appeared in satisfactory condition to the unaided eye. Among the liners in poorer condition, removal of existing surface deposits sometimes revealed definite checking of the rubber. When the swollen, stretched, or "tacky" condition existed, only slight improvement resulted from the cleaning treatment.

On microscopic examination all used liners were found to have definite breakdown of the inner surface of the rubber. This deterioration usually was more marked in the upper half of the liners and varied in type and degree. There was no close relationship between microscopic condition and macroscopic appearance. Many of the better-appearing used liners had as much or more microscopic breakdown than some of the more unsatisfactory liners. The most common condition was cracking, ranging from a shallow network to intense

breaking of the surface. In addition, some surfaces appeared crumbled and disintegrated. Others were pitted and spongelike. Surfaces that were rough to the touch usually appeared frayed and ragged. Where tacky areas were found in liners, the microscopic appearance suggested a moist viscous condition, and the surface was irregular and pitted but showed less cracking than did nontacky rubber. Where film deposits were present, they appeared, microscopically, as a gravel-like condition. After lye and organic acid treatments, the extraneous matter was removed or markedly reduced, and breakdown of the rubber usually was more obvious under microscopic examination. From the limited information obtained regarding the care of the rubber on the farm, there appeared to be no marked relation between the farm sanitizing and storage practices and the nature and extent of the surface breakdown in the rubber. However, when liners were stored in chlorine solution, checking of the rubber was the most obvious. Examples of the microscopic appearance of the inner surfaces of the used liners are shown in Figure 1. In photomicrographs C and D, bristles from a teat-cup liner brush are shown to indicate the comparative size of the cracks in the rubber. In photomicrograph C, the largest cracks shown were visible macroscopically as light checking.

In almost all new liners, microscopic examination showed an unbroken interior surface. No porous structure could be detected at the 85 × magnification employed. With some brands the surface was smooth, and in others slight ridges, with no breaks, were evident (Figure 2). The contrast between new and used liners, even when the latter were seemingly in good condition, was striking. Some new liners were obtained that showed early stages of cracking or other imperfections, such as breaks and pockets in the surface, with foreign particles loosely embedded in the rubber.

TABLE 1

Summary of bacterial counts on used and new liners after experimental contamination

	No. of trials [a]	Different used liners involved	No. of liner comparisons	Log av. count per ml. water rinse per liner		Ratio A/B
				A Used liners	B New liners	
All comparisons	26	52	104	12,300	650	19
Grouping according to extent of contamination						
Higher contamination	10	32	40	97,400	5,060	19
Lower contamination	16	20	64	3,400	180	19
Grouping according to condition of used liners [b]						
Better liners	17	32	68	12,700	716	18
Worse liners	9	20	36	11,700	542	21

[a] In each trial, counts were made individually on 4 used liners compared with 4 new liners contaminated from the same lot of milk. However, each trial involved a different lot of milk of different quality.

[b] Empirically classified according to macroscopic condition.

Effect of deterioration of the inner surface of the liner on susceptibility to contamination. When new and used liners were experimentally contaminated in the laboratory under the same conditions, the bacterial counts on the used liners were markedly higher in every instance. A summary of the results of 104 comparisons of 52 different used liners with 24 new liners is given in Table 1. The log average count on the used liners for all trials was 19 times as great as the log average count on the new liners. The actual counts on individual used liners, in sets of four, ranged from three to 92 times as great as the corresponding counts on new liners. A considerable range of contamination was obtained in the different trials since different lots of milk were used in each trial and no effort was made to standardize the degree of contamination. However, when the trials were grouped on the basis of higher and lower contamination, the ratios of log average counts on the used liners to the log average counts on the new liners were the same for each group.

Since the primary concern of the investigation was with the used liners which appeared to be in satisfactory macroscopic condition, the data also were grouped

TABLE 2

Examples of bacterial counts on used and new liners after experimental contamination from the same lots of milk [a]

Trial	Liner set	Liner condition [b]	Plate counts per ml. water rinse per liner [c]		Ratio A/B [d]
			A Used liners	B New liners	
1	A	Good	60,000	12,000	
			120,000	12,000	
			500,000	15,000	
			500,000	9,000	25
2	B	Fairly good	120	5	
			900	7	
			160	10	
			120	5	48
3	C	Poor	96,000	2,300	
			97,000	6,400	
			120,000	3,800	
			130,000	3,300	28
4	D	Poor	2,400	300	
			2,500	120	
			13,000	140	
			6,000	75	38
5	E	Fairly good	2,500	650	
			4,500	450	
			7,200	600	
			2,400	250	8

[a] Each trial involved a different lot of milk of different quality.
[b] General macroscopic condition of used liners after acid and lye cleaning.
[c] Bacteriological examinations made by the pulsation-vacuum procedure for individual liners; 35 ml. water rinse used per liner.
[d] Ratio of total of the 4 counts on used liners to total of the 4 counts on new liners.

on the basis of the better and worse liners. From the log average counts of each group it is evident that the liners appearing in less satisfactory condition were only slightly more susceptible to contamination than were liners that were considered to be reasonably satisfactory. This relationship was in harmony with the observations made microscopically.

Individual data from five of the foregoing trials are presented in Table 2 as examples of the results obtained. It may be seen that, within each trial, individual counts on the four used liners in a set usually fall in the same general range, and counts on the corresponding new liners are even more consistent and in a much lower range.

Comparisons under practical operating conditions. When contamination of the liners occurred under routine practical conditions of operation, bacterial counts on the used liners were again much higher than on the new liners at each examination. In trial 1 (Table 3) the greatest proportional difference was obtained at the initial examination after the liners had been on test 6 days. At this stage, the total of the four counts on the used liners was 55 times as great as the corresponding total for the four new liners. The proportional differences

TABLE 3

Bacterial counts on 4 used liners and 4 new liners after operation under the same practical conditions.[a]

Trial 1

Days in operation with 40 cows	Plate counts per ml. of water rinse per liner [b]		Ratio A/B [c]
	A Used liners	B New liners	
6	300	90	
	1,800	100	
	4,000	110	
	15,000	80	55
12	4,000	800	
	2,500	70	
	4,200	100	
	15,000	90	24
14	1,300	200	
	6,000	100	
	3,500	400	
	4,500	500	13
16	20,000	5,000	
	150[d]	600	
	8,000	300	
	1,200[d]	160	5

[a] Before making bacteriological examinations, liners were flush-washed in the laboratory as described under "Methods."

[b] Bacteriological examinations made by the pulsation-vacuum procedure for individual liners; 35 ml. water rinse used per liner.

[c] Ratio of total of the 4 counts on used liners to total of the 4 counts on new liners.

[d] These were newer liners replacing those that developed breaks in stem.

decreased in subsequent examinations. In the last period the change in relationship was due largely to discrepancies in counts on individual liners. In this regard it was noted that counts on individual liners in each set were less uniform than when experimental contamination was employed.

Results of a second trial using a different type of liner and with more examination periods are presented in Table 4. In the last two periods the liners were

TABLE 4

Bacterial counts on 4 used liners and 4 new liners after operation under the same practical conditions.[a]

Trial 2 (different type liners than trial 1)

Period in operation with 10 cows	Plate counts per ml. of water rinse per liner [b]		Ratio A/B [c]
	A Used liners	B New liners	
1 milking	2,500	95	
	2,200	115	
	3,500	400	
	6,000	220	17
2 milkings	4,000	300	
	2,000	180	
	1,200	250	
	5,000	500	10
3 milkings	6,000	12	
	600	20	
	500	45	
	1,500	35	77
4 milkings	48,000	1,100	
	2,000	900	
	5,000	700	
	18,000	400	24
6 d.	4,500	180	
	800	20	
	2,800	90	
	3,200	70	31
8 d.	5,500	100	
	1,600	55	
	1,100	45	
	8,000	42	67
12 d.	1,000	16	
	450	7	
	600	3	
	15,000	7	516
14 d.	12,000	130	
	1,800	100	
	1,000	130	
	50,000	110	138

[a] Before making bacteriological examination, liners were flush-washed in the laboratory as described under "Methods" except in the last two periods where sanitizing was done under routine practical conditions.

[b] Bacteriological examinations were made by the pulsation-vacuum procedure for individual liners: 35 ml. water rinse used per liner.

[c] Ratio of total of the 4 counts on used liners to total of the 4 counts on new liners.

examined after the routine sanitizing procedure employed at the dairy barn.[4] In all cases the bacterial counts again were much higher for the used liners than for the new liners, ranging from 11 to more than 500 times as great in sets of four liners. The greatest differences occurred in the last two periods when the liners had been sanitized under practical conditions. In these two periods more variation occurred in the bacterial counts of the individual used liners than during the previous periods. Contrary to the previous trial, there was no tendency for the proportional differences to become less as the trial progressed. It is interesting to note that, even during the first milking period, the used liners acquired much heavier contamination than the new liners.

DISCUSSION

The high counts obtained on used liners demonstrate how discrepancies may arise in comparative bacteriological studies of milking machines where microscopic condition of the liners has not been considered. When all the liners employed are new, the problem is minimized. However, although the use of new liners is satisfactory for certain sanitation studies, it is obvious that results may not always apply to used liners. For example, simplified cleaning methods might provide effective sanitation with new rubber, but more thorough procedures seem indicated for liners with inconspicuous surface breakdown.

It was evident from this study that surface deterioration in liners may reach the stage where susceptibility to contamination is greatly increased, even though the liners appear to be in suitable condition for effective milking operations. The problem would appear to merit the attention of the manufacturers of teat-cup liners. Assuming that some surface deterioration is unavoidable, it seems essential that cleaning procedures be developed with due consideration to the surface characteristics of the deteriorated surface. For example, with such surfaces as shown in Figure 1, the use of a brush would appear to be inadequate for removing soil and bacteria protected by irregularities of the rubber surface. Moreover, such brushing might tear further the surfaces of the rubber and lead to greater susceptibility to contamination. Likewise, in the utilization of cleaning and sterilizing agents, consideration should be given to the differences in surface characteristics of used and new rubber.

It is conceivable that incipient surface deterioration of teat-cup liners may be a factor in mastitis control problems. Since large numbers of bacteria may be entrapped by the finely cracked and eroded surfaces, the possibility for the spread of organisms causing mastitis is enhanced. It is conceivable that chlorine solution dipping and other rinse techniques used on liners between milkings of consecutive cows would be less effective with liners having surface breakdown than with new liners. In this respect, Spurgeon *et al.* (6) have reported that the

[4] In the barn procedure, after an initial tepid water flushing of the units, the liners were removed from the shells and brush washed in detergent solution at about 140° F. They then were rinsed in warm water containing chlorine and stored dry.

effectiveness of germicides was markedly reduced when old, cracked liners were used.

SUMMARY AND CONCLUSIONS

A microscopic and bacteriological study was conducted on used teat-cup liners in comparison with new liners to determine the effect of inconspicuous surface deterioration on susceptibility to contamination. Fifty-two used liners in sets of four were obtained from five farm sources. Most of the liners were currently in service and 32 were considered to be in reasonably satisfactory physical condition as indicated by macroscopic examination. Microscopic examination showed breakdown of the interior surface of varying types and extent in all used liners. There was no marked relationship between microscopic and macroscopic appearance.

Bacterial counts on liners having microscopic deterioration always were markedly higher than on new liners contaminated under similar conditions. In 104 comparisons with experimental contamination, the log average count for the used liners was 19 times as great as the log average count for new liners. Used liners appearing to be in satisfactory condition were only slightly less susceptible to contamination than were apparently less satisfactory liners. In two trials, with contamination occurring under practical conditions, bacterial counts were from five to more than 500 times as great for sets of four used liners as for sets of four new liners.

It is evident that teat-cup liners in the early stages of surface deterioration are more susceptible to bacterial contamination than are new liners, even though they often appear to be almost similar in general physical condition. Such a relationship necessitates consideration in milking machine sanitation problems. Furthermore, since the liners may still function satisfactorily in the milking operation, the problem appears to merit the attention of the liner manufacturers.

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A HIGH ROUGHAGE SYSTEM FOR RAISING CALVES BASED ON THE EARLY DEVELOPMENT OF RUMEN FUNCTION. I. EFFECT OF VARIATIONS IN THE RATION ON GROWTH, FEED CONSUMPTION, AND UTILIZATION

J. W. HIBBS, W. D. POUNDEN, AND H. R. CONRAD
Ohio Agricultural Experiment Station, Wooster

Economy in feeding the ruminant is based fundamentally on taking full advantage of rumen function, with particular emphasis on the digestion of roughages and the synthesis of high quality proteins and vitamins. In efforts to apply this axiom to calf feeding, preliminary work indicated that mature type rumen function could be established in young calves much earlier than was generally thought feasible (9, 10). Young calves receiving only limited quantities of milk were raised satisfactorily on a high-roughage, low-cost ration similar to that usually provided older cattle. The ration consisted of approximately two parts good quality mixed legume-grass hay and one part of a simple 14.5 per cent total protein grain concentrate mixture. In the preliminary experiments considerable attention was given to management factors which were observed to influence the health and the early development of rumen function of young calves, including rumen inoculations (11, 12, 13).

The purpose of the following experiments was to study some variations which could be made in this basic high-roughage feeding system in order to obtain maximum efficiency of feed utilization and low cost along with normal growth and health. A simple grain concentrate mixture was compared with a complex calf starter ration fed in a 2:1 hay-grain ratio with two types of hay. Also, the effect of feeding whole milk for either 7 or 9 weeks with and without vitamin B_{12} concentrate using a 4:1 hay-grain ratio was studied. Comparisons were made also of differences in calf performance and efficiency of feed utilization to 6 months of age between two different hay-grain ratios used in these experiments.

During past years several systems of calf feeding have been developed which were aimed at a reduction in milk consumption. In these systems, notably the Cornell and New Jersey systems, the approach has been to use a calf "starter" concentrate mixture containing milk by-products and other sources of animal protein and vitamins as a substitute for whole milk. In contrast, the aim in the high-roughage system has been to develop mature type rumen function as early as possible and in so doing eliminate the need for a calf "starter" in meeting the nutritional requirements of the calf. When rumen function is adequately developed, the products of rumen digestion and synthesis may be considered as a natural replacement for milk.

Considerable evidence has been accumulated which indicates that normal health is difficult to maintain in young ruminants without adequate roughage

Received for publication January 13, 1953.

in the ration. As early as 1897, Davenport (2) showed that abnormal conditions resulting from a diet of milk alone could be corrected by feeding hay or straw. Similar results were reported by McCandlish (6) in 1923. It has been shown further that the provision of good quality hay in the ration of calves during the first few days effectively prevented the formation of hair and fiber balls in their rumens (13). Limiting the amount and also the protein content of the grain fed with high quality legume hay lowered the incidence of diarrhea in calves (13).

Rough hair coats and pot-bellies were observed in calves fed a high roughage ration and kept segregated from older animals, which prevented natural transfer of the usual types of rumen microorganisms (11, 12). Rumen inoculations (11) using freshly obtained cud material taken from older cattle and containing masses of typical rumen microorganisms were effective in overcoming the detrimental effects of the absence of these microorganisms. Evidence has been obtained which indicated that this beneficial effect was elicited, at least in part, by increased roughage digestion and vitamin synthesis (1, 3) by the rumen microorganisms transferred in the cud material.

The principal factor, in addition to segregation, that was found to inhibit the establishment of several varieties of rumen microorganisms characteristically present in the rumens of mature dairy cattle was the ingestion of grain concentrates in excess of the hay consumed (9, 10). When grain feeding was limited to an amount less than that of the hay being eaten, mature type rumen function could be established readily in young rumen-inoculated calves before the end of a 7-week milk feeding period.

EXPERIMENTAL PROCEDURE AND RESULTS

Experiment 1: Simple vs. complex grain with alfalfa or mixed hay fed in the ratio of two parts hay to one part grain. This experiment was designed to determine the relative value of a simple 14.5 per cent total protein grain mixture compared to a complex 25 per cent total protein, pelleted, commercial calf starter when fed as the grain concentrate mixture in the high-roughage system.

In addition to this comparison, a test was made of calf preference for second cutting alfalfa (average total protein 14.4 per cent) or second cutting clover-timothy hay (average total protein 10.6 per cent) fed free choice in divided mangers. Calves fed both hays in this way were compared with another group fed only third cutting alfalfa hay (average total protein 15.0 per cent).

Calves were allowed to nurse until their dams were milked out at 12 to 24 hours postpartum. At the first milking or as soon thereafter as possible sufficient colostrum was saved to complete 3 days of feeding at the daily rate of 0.9 lb. colostrum per 10 lb. of birth weight.

The 17 Jersey calves used were placed on experiment after the colostrum feeding period at 3 days of age and divided into four groups according to the following experimental plan:

No. of calves	Grain concentrate mixture	Type of hay fed
3 ♀ – 2 ♂	simple	alfalfa-clover-timothy, 2nd cutting
3 ♀ – 2 ♂	complex	alfalfa-clover-timothy, 2nd cutting
3 ♀	simple	alfalfa, 3rd cutting
4 ♀	complex	alfalfa, 3rd cutting

The simple grain concentrate mixture and the complex calf starter were of the following compositions:

	Simple *(lb.)*	Complex *(lb.)*
Ground shelled corn	400	198
Ground oats	300	50
Wheat bran	100	50
Soybean oil meal	100	325
Linseed oil meal	.....	100
Alfalfa meal	.....	50
Fish meal	.....	50
Dried whey	.....	150
Ground limestone	.....	10
Steamed bonemeal	.....	10
Iodized salt	9	5
Vitamin A feeding oil	.....	1
D-activated animal sterol	.....	0.5
Anise oil	.....	0.5
Total	909	1,000.0

The experiment was continued to 10 weeks of age, when the male calves were slaughtered. Whole milk was fed from open buckets for 6 weeks at the daily rate of 0.9 lb. per 10 lb. of birth weight and gradually reduced during the 7th week. No milk was fed after the 7th week. Hay of the type assigned was fed free choice from the start. Fresh hay was fed each day, allowing for 15 to 20 per cent refusal. Feeding of the respective grain concentrate mixtures was begun during the 6th week at the rate of ¼ lb. daily for Jerseys and ½ lb. daily for Holsteins. After the 6th week grain feeding was limited to approximately one-half the amount of hay being consumed daily. Adjustments in grain feeding were made at weekly intervals, or less, as needed to maintain the desired 2:1 proportion. All calves were given rumen inoculations on the 5th, 10th, and 15th days of age. Water, salt, and bonemeal were supplied free choice at all times.

The calves were weighed weekly, and daily records were kept of hay and grain consumption. Feed consumption, growth, and the efficiency of feed utilization, which was measured as calculated total digestible nutrients (TDN) and calculated net energy (NE) per pound of gain, over the 10-week period are summarized in Table 1.

TABLE 1

Performance of Jersey calves fed a simple vs. complex grain mixture with alfalfa or mixed hay to 10 weeks of age

	Mixed hay		Alfalfa hay					
	Simple grain	Complex grain	Simple grain	Complex grain	Simple grain	Complex grain	Mixed hay	Alfalfa hay
No. of calves	5	5	3	4	8	9	10	7
Whole milk *(lb.)*	212.4	207.5	218.7	227.7	214.8	216.5	210.0	223.8
Alfalfa hay *(lb.)*	41.8	37.6	56.0	52.1	47.1	44.0	39.7	53.8
Clover-timothy hay *(lb.)*	32.0	32.2	.....	.....	20.0	17.9	32.1	.....
Total hay *(lb.)*	73.8	69.8	56.0	52.1	67.1	61.9	71.8	53.8
Complex grain *(lb.)*	.....	21.5	.....	23.1	.....	22.2	10.8	13.2
Simple grain *(lb.)*	28.8	.....	20.6	.....	25.7	.....	14.4	8.8
Birth weight *(lb.)*	57.0	51.0	46.0	55.0	53.0	53.0	54.0	51.0
Weight at 10 wk. *(lb.)*	94.0	85.0	90.0	101.0	95.0	92.0	91.5	96.3
Gain, 10 wk. *(lb.)*	37.0	34.0	44.0	43.0	39.3	38.0	35.2	43.3
Gain, 10 wk. *(%)*	71.9	66.6	95.6	83.7	79.0	73.6	69.3	88.8
TDN/lb. gain *(lb.)*[a]	2.49	2.64	1.83	1.83	2.24	2.28	2.56	1.83
NE/lb. gain *(Therms)*[a]	2.66	2.75	1.94	1.97	2.37	2.45	2.70	1.96

[a] TDN and NE calculated from Morrison's (8) appendix, Tables 1 and 2.

It will be noted that when clover-timothy hay was offered along with the alfalfa hay, free choice, considerable clover-timothy hay was eaten, indicating a desire for variety. The calves ate more total hay when offered both kinds than when only alfalfa was fed.

In this experiment the calves fed only third cutting alfalfa hay gained more efficiently and at lower cost [1] than did the calves fed mixed hay, even though they ate less total hay. The differences in TDN and NE per pound of gain were statistically significant ($P < 0.05$). The eight calves fed the simple grain concentrate mixture gained as well as the nine calves fed the complex calf starter mixture during the 10 weeks. Thus, there was no advantage in feeding the complex grain mixture in this system with either of the types of hay used. Costs were slightly higher when the complex grain mixture was fed, but efficiency of feed utilization was equal between the groups.

The 13 heifers used in this experiment were continued on the 2:1 ratio of hay to grain to 6 months of age. They were fed alike after the 10th week except that a few heifers received the complex starter mixture for a short time beyond 10 weeks. Table 3 shows the performance of these heifers over the entire 6-month period as measured by feed consumption, growth, TDN and NE per pound of gain. Average growth over the 6-month period was approximately equal to the

[1] Costs as referred to in this paper are based on prevailing market prices for the various feeds.

Ragsdale standard (14). TDN per pound of gain averaged 2.72 lb., and NE per pound of gain averaged 2.56 therms.

In order to obtain information on the efficiency of feed utilization of the heifer calves fed the 2:1 hay-grain ratio uncomplicated by milk feeding and different types of hay and grain concentrate, TDN and NE per pound of gain were calculated for the arbitrarily selected 9-week period of 18 to 26 weeks of

TABLE 2

Performance of Jersey calves to 12 weeks of age fed a ground high roughage mixture with or without vitamin B_{12} with milk for 7 vs. 9 weeks

	Control		Vitamin B_{12}					
	Milk 7 wk.	Milk 9 wk.	Milk 7 wk.	Milk 9 wk.	Control	Vitamin B_{12}	Milk 7 wk.	Milk 9 wk.
No. of calves	7	5	6	6	12	12	13	11
Whole milk *(lb.)*	272.0	279.0	271.0	343.0	275.0	307.0	273.0	314.0
Dry feed mixture *(lb.)*	173.0	143.0	187.0	117.0	161.0	152.0	179.0	129.0
Birth weight *(lb.)*	60.0	47.0	60.0	55.0	54.6	57.5	60.0	51.4
Weight at 12 wk. *(lb.)*	121.0	104.0	124.0	110.0	114.0	117.0	122.4	107.3
Gain, 12 wk.*(lb.)*	61.0	57.0	64.0	55.0	59.3	54.5	62.4	55.9
Gain, 12 wk. *(%)*	102.0	121.0	107.0	100.0	109.9	103.5	104.3	108.8
TDN/lb. gain *(lb.)*[a]	2.29	2.17	2.30	2.17	2.24	2.24	2.30	2.17
NE/lb. gain, *(Therms)*[a]	2.29	2.26	2.27	2.25	2.28	2.26	2.28	2.25

[a] TDN and NE calculated from Morrison's (8) appendix, Tables 1 and 2.

TABLE 3

Performance of Jersey heifers raised to 6 months of age on different hay, grain ratios.

	2:1 Ratio[a]	4:1 Ratio[b]
No. of heifers	13	6
Whole milk *(lb.)*	219.0	337.0[d]
Alfalfa hay *(lb.)*	530.0	698.0
Clover-timothy hay *(lb.)*	18.0	58.0
Alfalfa meal *(lb.)*	.....	14.0
Total hay *(lb.)*	548.0	770.0
Simple grain *(lb.)*	227.0	201.0
Complex grain *(lb.)*	33.0	.....
Total grain *(lb.)*	261.0	201.0
Birth weight *(lb.)*	52.0	59.0
Weight 6 mo. *(lb.)*	237.0	248.0
Gain, 6 mo. *(lb.)*	185.0	189.0
Gain, 6 mo. *(%)*	356.0	320.0
TDN/lb. gain, 6 mo. *(lb.)*[c]	2.72	3.17
NE/lb. gain, 6 mo. *(therms)*[c]	2.56	2.87
TDN/lb. gain, 18-26 wk. *(lb.)*[c]	3.18	3.70
NE/lb. gain, 18-26 wk. *(therms)*[c]	2.86	3.29

[a] These heifers were on Experiment 1 to 10 wk. of age.
[b] These heifers were on Experiment 2 to 12 wk. of age.
[c] TDN and NE calculated from Morrison's (8) appendix, Tables 1 and 2.
[d] Four of these calves were fed milk for 9 wk.

age. During this period all the heifer calves were being fed alike on the 2:1 ratio and were considered to be relatively free from any possible influences that might have persisted from differences in treatment prior to 10 weeks of age. Average

TDN and NE per pound of gain for the 9 week period also are shown in Table 3. Individual differences in TDN per pound of gain varied from 2.50 lb. to 3.69 lb., with an average of 3.18 lb. NE per pound of gain ranged from 2.34 therms to 3.29 therms, with an average of 2.86 therms.

Experiment 2: Milk 9 weeks vs. 7 weeks; vitamin B_{12} vs. no vitamin B_{12} fed with 4:1 ratio of hay to grain. Twenty-four Jersey calves were used in this experiment. Colostrum feeding was carried out as in experiment 1 except that 1 lb. instead of 0.9 lb. of colostrum per 10 lb. of birth weight was fed daily.

The calves were assigned to their respective groups and placed in individual pens when removed from their dams. Beginning on the 4th day, whole milk was fed at the daily rate of approximately 1 lb. per 10 lb. of birth weight except during the last week, when the milk was gradually reduced to zero.

As soon as the calves were taken from their dams, a high-roughage, dry-feed mixture was offered free choice. This mixture consisted of four parts coarsely ground hay, 1 part a simple grain concentrate mixture (same as that described in experiment 1), and one-half part alfalfa meal. The alfalfa meal was added mainly for the purpose of increasing the carotenoid content of the mixture to about 20 parts per million. The hay in the mixture was one-half early-cut timothy and one-half third-cutting alfalfa. The complete dry-feed mixture contained an average of 14.5 per cent total protein. Steamed bonemeal and salt were kept before the calves at all times in separate containers, and water was available *ad lib.*

All calves were inoculated with cud material from older cattle in the herd at the end of the 1st week and once weekly thereafter for 6 weeks.

Twelve calves, six of which were fed whole milk for 7 weeks (2 ♂, 4 ♀) and the other six for 9 weeks (3 ♂, 3 ♀), were given a vitamin B_{12} supplement (Merck's APF supplement No. 3) containing 12.5 mg. of vitamin B_{12} per pound. The supplement was fed at the rate of 1.3 g. (35.8 γ of vitamin B_{12}) per day added to the milk, except during the last week of the milk-feeding period. Beginning 1 week before the end of the milk-feeding period and continuing to 12 weeks of age, 1.3 g. of the APF supplement was included in each pound of the dry feed mixture. Twelve similarly fed control calves were given no APF supplement. Seven of the control calves (4 ♂, 3 ♀) were fed milk for 7 weeks and 5 (2 ♂, 3 ♀) were fed milk for 9 weeks.

All calves were weighed weekly, and daily records of feed consumption were kept during the 12-week experimental period.

The performance of the calves based on feed consumption, growth, and the efficiency of feed utilization measured by TDN and NE per pound of gain is shown in Table 2. Average weekly changes in body weight compared to the Ragsdale standard (14) are shown in Figure 1. Figure 2 shows the average weekly feed consumption in pounds of the 4:1 hay-grain mixture per week.

No differences in performance were attributed to feeding the vitamin B_{12} supplement.

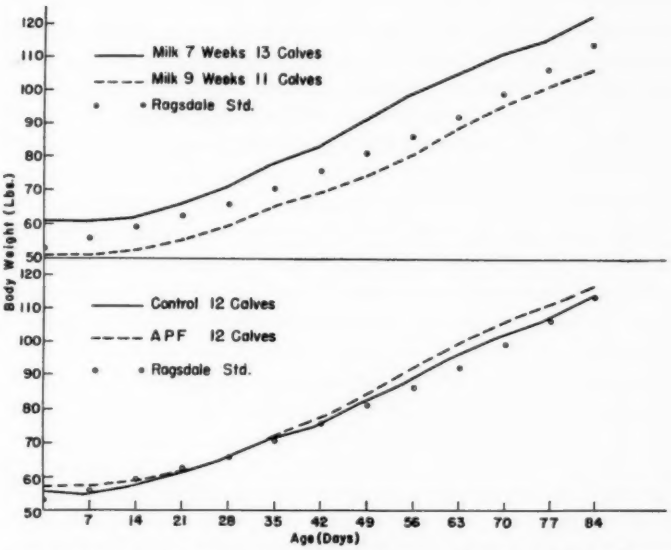

FIG. 1. The influence of feeding vitamin B_{12} supplement (Merck's APF Supplement No. 3) and whole milk fed for 7 *vs.* 9 weeks on the growth of Jersey calves fed a 4:1 hay to grain ratio (Experiment No. 2).

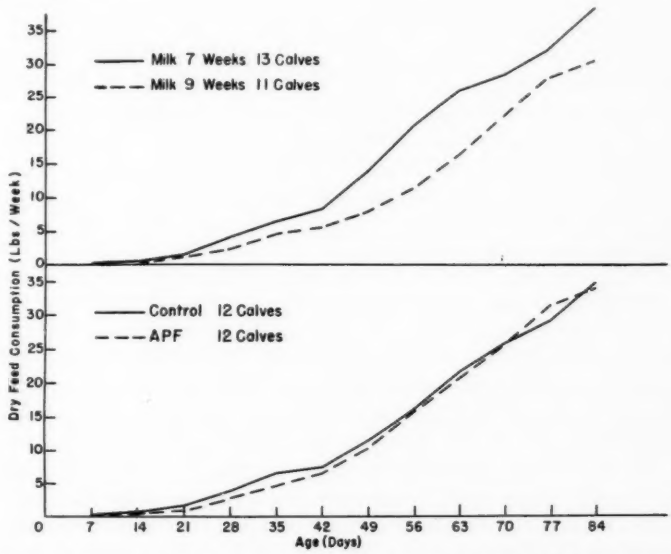

FIG. 2. The influence of feeding vitamin B_{12} supplement (Merck's APF Supplement No. 3) and whole milk for 7 *vs.* 9 weeks on the weekly dry feed consumption by Jersey calves fed a 4:1 ratio of hay to grain (Experiment No. 2).

No differences in performance were found between calves fed milk for 7 weeks and those fed milk for 9 weeks except that the 9-week milk-fed calves showed a higher average cost and some suppression in feed intake after 6 weeks of age compared to calves fed milk for 7 weeks. The difference in cost was due to the higher milk intake in the 9-week milk-fed calves; this intake would have been even higher if the calves in this group had been as heavy at birth as the calves fed milk for 7 weeks.

Six of the heifers in this experiment were continued on loose alfalfa hay, second cutting, and the simple grain mixture fed in the ratio of four parts hay to one part grain from the end of the 12th week to 6 months of age.

The performance of the heifers fed the 4:1 ratio of hay to grain is compared with that of heifers fed the 2:1 ratio (experiment 1) for the entire 6-month period (Table 3).

The heifers fed the 4:1 ratio of hay to grain consumed more milk, more hay, and less grain than the heifers fed the 2:1 ratio. In spite of the differences in feed intake the differences in growth were not large. Partly because of the extra milk consumed, the feed cost was greater for the heifers fed the 4:1 hay-grain ratio than for those fed at the ratio of 2:1. TDN per pound of gain averaged 3.17 lb. and NE per pound of gain averaged 2.87 therms, compared to 2.72 lb. and 2.56 therms, respectively, for the heifers fed the 2:1 ratio. Thus, it appeared that the heifers fed the 2:1 ratio utilized their feed more efficiently than did those fed the 4:1 ratio.

TDN and NE per pound of gain of the six heifers in experiment 2 were calculated for the 9-week period, 18-26 weeks (Table 3). Average TDN and NE per pound of gain were, respectively, 3.70 lb. and 3.29 therms, with a range in TDN from 3.10 to 4.08 lb. and in NE from 2.73 to 3.59 therms. It was found that, although there was little difference in gain during the 9-week period (2:1 heifers gained 91 lb. and the 4:1 heifers 94 lb.), the heifers fed the 2:1 ratio made their gains more efficiently and at lower cost than the heifers fed the 4:1 ratio. Differences in efficiency of feed utilization between the heifers fed the 2:1 and 4:1 ratios under these conditions were found to be statistically significant, TDN ($P < 0.01$), NE ($P < 0.02$).

DISCUSSION

Using the system described, rumen function and capacity were sufficiently developed during the 7-week milk-feeding period to permit satisfactory growth and development thereafter on a ration consisting largely of good roughage with limited quantities of a simple, low-cost, all-vegetable protein grain concentrate mixture. No advantage was found in using a complex, high-protein grain concentrate compared to a simple, low-protein grain concentrate. The fact that no break in the growth curves resulted when milk was removed from the diet is further evidence of adequate rumen development during the milk feeding priod. This was indicated by the weekly changes in body weight for the first 12 weeks in the calves fed whole milk for 7 weeks in experiment 2, compared to the Ragsdale

standard (14), Figure 1. Microscopic examinations of fresh preparations and Gram stains of the rumen liquor obtained by stomach tube made at frequent intervals, using the indicator method (9, 10), also showed that mature type rumen function was well established during the period of 4 to 6 weeks of age, depending upon the eating habits of the calf.

Table 1 shows that the alfalfa hay used resulted in greater growth and more efficient utilization of feed than did the clover-timothy hay. However, in experiment 2 the mixed hay used provided for good growth. This emphasizes the importance of the quality and acceptability of the roughage in this system.

No benefit was demonstrated from feeding the vitamin B_{12} supplement. In a later experiment (3) involving Holstein calves, no effect on growth was found from feeding the same vitamin B_{12} supplement.

No advantage was found to result from extending the milk feeding period beyond 7 weeks. On a practical basis, however, milk should not be taken out of the ration if a calf is not yet eating a liberal amount of dry feed. It is also possible that more milk fed after the first 2 weeks could have provided for faster growth without delaying hay consumption and rumen development. Although not reported here, the results of blood vitamin A analyses indicated that if limited whole milk feeding is practiced without any vitamin A supplement in the concentrate mixture, some source of supplemental vitamin A should be provided during the milk feeding period, especially when the milk fed is from cows maintained under barn-feeding conditions.

Despite the marked differences in relative amounts of feed consumed, including the amount of milk fed, gains in body weight were not appreciably different between the heifer calves started in experiment 1 or 2 and continued to 6 months of age on either the 2:1 or 4:1 ratio of hay to grain respectively, as shown in Table 3. The average TDN and NE per pound of gain for the group fed the 4:1 ratio for the entire 6-month period were considerably higher than those of the group fed the 2:1 ratio. This difference may have been influenced somewhat by the greater milk consumption of the 4:1 group.

The differences in average TDN and NE per lb. of gain between heifers fed the 2:1 and those fed the 4:1 ratios of hay to grain during the 9-week period, 18 to 26 weeks of age, uncomplicated by milk consumption, also are shown in Table 3. The efficiency of feed utilization was significantly lower in the group fed the 4:1 ratio than in the group fed the 2:1 ratio. The average difference in growth during this period, however, was only 3 lb.

It is of interest that the calves were able to develop sufficient extra capacity to consume and digest roughage so that when grain concentrate was fed at the lower level, 4:1, enough more hay could be consumed and digested to allow for growth equal to that of the group fed the 2:1 ratio. This was possible in spite of the fact that the TDN and NE were utilized less efficiently in the group fed the 4:1 ratio.

Further work is in progress to determine differences in performance of calves during the first 12 weeks when fed different proportions of hay to grain, using the same kind of hay, a comparison which was not possible in these experiments.

The relative inefficiency of feed utilization in the calves fed the 4:1 ratio may be due, at least in part, to the loss of energy due to heat increment coincident with the rumen production of a larger proportion of acetic acid in relation to other volatile fatty acids (3, 7). Another factor probably involved in the relative inefficiency of the 4:1 ratio group is indicated by the finding that calves fed a 4:1 ratio of hay to grain retained less nitrogen than those fed either a 3:2 or 2:3 ratio under controlled feeding conditions (3). This supports the evidence of Kuhn *et al.* (4) and Lofgreen *et al.* (5) that nitrogen retention is dependent upon the level of energy in the ration of cattle. On this basis, the 4:1 ratio of hay to grain probably resulted in less nitrogen retention than the 2:1 ratio as a result of the lower energy content.

SUMMARY AND CONCLUSIONS

A low-cost, high-roughage system of raising calves based on the early development of mature type rumen function is described. This system utilized a limited amount of whole milk and mostly home grown feeds, including large amounts of good quality roughage and limited amounts of grain concentrates. Suitable conditions thus were provided for the early establishment of mature type rumen microorganisms. Rumen microorganisms were supplied through rumen inoculations, using fresh warm cud material taken from healthy older cattle.

The results of experiments are presented in which this basic high roughage feeding system was varied in an effort to obtain maximum efficiency of feed utilization, satisfactory growth, and lowest cost and at the same time take full advantage of the inherent capacities of the calf to digest roughage and synthesize nutrients in the rumen.

In this system there was no advantage in feeding a complex calf starter ration compared to a simple, low-protein grain concentrate mixture. This is attributed to the fact that rumen function was sufficiently developed in most calves by 7 weeks of age to permit all the milk and other sources of animal protein to be taken from the ration without decreasing growth.

Also, it was shown that a high proportion of legume (alfalfa) in the hay is desirable. However, considerable amounts of young grass hay were readily eaten by the calves when it was made available. High quality and acceptability of the roughage available is fundamental to the success of feeding calves on this system.

No benefit was found from including vitamin B_{12} supplement (Merck's APF supplement No. 3) in the ration of the calves raised on this system.

The efficiency of feed utilization as measured by TDN and NE per pound of gain of the 2:1 hay-grain ratio was greater than that of the 4:1 ratio. However, very little difference in growth was found when one ratio was compared with the other under the existing conditions.

The results of these experiments and some of their implications are discussed in relation to recent developments in the physiology of rumen function.

ACKNOWLEDGMENTS

The authors wish to express appreciation to J. E. Tate, H. D. Miller, and R. L. Johnson for their help in conducting these experiments.

The calf starter used was generously supplied by the Ohio Farm Bureau Coop. Assoc., Feed Division, Columbus, Ohio, and the vitamin B_{12} supplement by Merck and Co., Rahway, N. J.

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THE EFFECTS OF FREEZING BOVINE SPERMATOZOA IN EXTENDERS CONTAINING ANTIBACTERIAL AGENTS

H. O. DUNN, G. L. LARSON, AND E. L. WILLETT
American Foundation for the Study of Genetics, Madison, Wisconsin

Investigations in England by Parkes (6, 7), Polge and associates (8, 9, 10, 11, 12), and Smith and Polge (13, 14) have revealed promising new developments in methods for freezing and storing mammalian and avian spermatozoa at −79° C. Preliminary breeding experiments (10, 11) have resulted in pregnancies in cattle inseminated with spermatozoa stored as long as 20 weeks at −79° C., and calves which appeared to be normal have been produced from frozen semen stored for shorter periods (9). These investigators concluded that the important steps in the procedure are: (a) to allow the semen in yolk-citrate to cool to 5° C. from 4 to 6 hours, (b) to add gradually during 30 minutes an equal volume of citrate buffer containing 20 per cent glycerol, (c) to equilibrate the semen and diluent for 12 to 20 hours at 5° C., and (d) to cool the preparation slowly from 5° C. to −15° C. at a rate not to exceed two degrees per minute, although below −15° C. the rate may be more rapid.

The British yolk-citrate extender for freezing bovine spermatozoa, according to the above reports, did not contain any of the antibacterial agents commonly used in this country for control of bacteria and preserving fertility of bovine semen. The present investigation was designed to study the effects of freezing spermatozoa in extenders containing different combinations of sulfanilamide, penicillin, and streptomycin.

EXPERIMENTAL

Viability of spermatozoa stored at −75° C. A 2^3 factorial experiment (2, 5) was conducted to determine the motility and revival rates of spermatozoa frozen to −75° C. in extenders containing the eight possible combinations of three antibacterial agents, each at two levels. One level was the absence of the particular antibacterial agent. The other level of each was 0.3 per cent sulfanilamide, 500 units of potassium penicillin-G, and 500 units of dihydrostreptomycin sulfate per milliliter of extender.

The basic extender consisted of egg yolk mixed with an equal volume of 2.9 per cent sodium citrate dihydrate. One extender contained no antibacterial agent. The other seven were prepared to contain initially either 0.3 per cent sulfanilamide, 1,000 units of penicillin per milliliter, 1,000 units of streptomycin per milliliter, or the different combinations of each. Tubes of 5-ml. portions of each extender were placed in a 400-ml. beaker filled with water at 30° C. before adding 0.25-ml. portions of semen to each tube. The initial extension rate was 1:20.

The extended semen samples and water bath were placed in a refrigerator and

Received for publication January 15, 1953.

allowed to cool slowly to 5° C. Four to 5 hours later, 5 ml. of 20-per cent glycerol (by volume) in citrate buffer, also cooled to 5° C., were added to each sample at the rate of 1 ml. every 6 minutes. It was necessary to add a glycerolated buffer containing 0.3 per cent sulfanilamide to the extenders containing sulfanilamide. As a result of this procedure, the final extenders contained 25 per cent egg yolk and 10 per cent glycerol, and the concentrations of penicillin and streptomycin were reduced to 500 units per milliliter.

A storage period of 12 to 20 hours at 5° C. for equilibration of the spermatozoa and glycerolated extender was allowed before the freezing was begun. Small test tubes (10 × 75 mm.) and pipettes were cooled to 5° C., and then 1-ml. portions of each sample were pipetted into tubes for freezing. The tubes were partially immersed in a shallow bath of ethyl alcohol at 5° C. in a Thermos vacuum vessel. Cooling from 5° C. to −15° C. over a period of 25 minutes was accomplished by periodic additions of small pieces of solid CO_2 to the alcohol bath. Below −15° C. the rate of cooling was more rapid.

Frozen samples were stored for 30 hours at approximately −75° C. in an alcohol bath in a large chest filled with solid CO_2. All samples of unfrozen semen were stored at 5° C. during the same 30-hour period in the respective eight treatments.

This experiment consisted of ten replicates, or semen samples, from three Holstein, four Guernsey, one Brown Swiss, and two Jersey bulls. Spermatozoan concentrations ranged from 18 to 47 million with a mean of 32 million per milliliter of extended semen as a result of the final extension rate of 1:40. This was the highest dilution rate tested by Polge *et al.* (11).

The frozen samples were thawed rapidly in a water bath at 30 to 38° C. (10). Estimations of per cent of motile spermatozoa in duplicate samples were made by two persons, and the mean was used as the motility of the sample. Estimations of the per cent of motile spermatozoa in the eight corresponding treatments stored at 5° C. were made in the same manner.

In this study, spermatozoan revival rates for the 80 samples were calculated according to the following formula:

$$\% \text{ revival} = \frac{\% \text{ motile after 30 hr. at } -75^\circ \text{ C.} \times 100}{\% \text{ motile after 48 hr. at } 5^\circ \text{ C.}}$$

More valid revival rates could have been obtained by estimating spermatozoan motility in each sample immediately before the freezing process.

Preliminary field experiment. In September, 1952, a limited number of inseminations were made to compare the fertility of frozen and unfrozen semen in extenders containing no antibacterial agents and in extenders containing sulfanilamide, penicillin, and streptomycin. The frozen samples were removed from the alcohol bath and transported in Thermos vacuum vessels containing solid CO_2. Each sample was thawed in lukewarm water at the time of insemination. Twelve technicians made the inseminations under the personal supervision of the authors.

Toxicity of sulfanilamide in frozen extenders. Two extenders containing 25 per cent yolk in sodium citrate buffer, one with and one without 0.3 per cent sulfanilamide, were frozen to – 65° C. by the method described above for freezing semen samples. Both extenders were thawed and warmed to 30° C. before addition of raw semen. A freshly prepared extender containing sulfanilamide served as an experimental control. Sixteen ejaculates from 15 bulls were sampled. Each sample was extended at a rate to give 12 million spermatozoa per milliliter and stored for 48 hours at 5° C. before estimations of motility were made.

RESULTS AND DISCUSSION

Viability of spermatozoa stored at – 75° C. The treatment means of per cent of motile spermatozoa in samples stored at 5° C. and – 75° C. and the corresponding average revival rates after the freezing and thawing process are shown in Table 1.

TABLE 1

The per cent of motile spermatozoa in 8 treatments after storage at 5° C. and – 75° C. and the per cent revival in each treatment after thawing (av. of 10 semen samples)

Antibacterial agents	Motility after storage at		Revival rates after thawing
	5° C.	– 75° C.	
	(%)	(%)	(%)
None	68	55	83
Sulfanilamide	67	32	48
Penicillin	66	48	76
Penicillin-sulfa	65	30	46
Streptomycin	65	52	80
Streptomycin-sulfa	64	32	50
Penicillin-streptomycin	67	45	67
Penicillin-streptomycin-sulfa	63	32	50

The data clearly indicate that the viability of spermatozoa stored at 5° C. was satisfactory in all treatments. However, in each treatment combination containing sulfanilamide, there was a decrease of 29 to 43 per cent in the average motility of spermatozoa stored at – 75° C. Similar reductions of revival rates were attributed to the presence of sulfanilamide. The revival rates in the two treatments containing no antibacterial agents and streptomycin alone correspond to the best revival rates reported by Polge and Rowson (12). The analysis of variance for treatment main effects and interactions revealed that the only significant treatment effect was due to the difference in the two levels, absence and presence, of sulfanilamide ($P < 0.001$).

These data suggest that sulfanilamide should not be included in extenders for freezing of bovine spermatozoa. The relative effects of penicillin and streptomycin on fertility of frozen semen should be investigated in field experiments, since the respective treatment effects on motility and revival rates were not significant ($P > 0.05$).

Preliminary breeding results. Forty-two inseminations were made with frozen semen during a period of 7 days starting 2 days after collection, and 138 insemi-

nations were made with the unfrozen semen on the 1st and 2nd day after collection. The 60- to 90-day per cent non-returns for the frozen and unfrozen semen from the one bull were 76 and 75, respectively, for the samples containing no antibacterial agents, and 81 and 33, respectively, for the samples containing all three antibacterial agents.

The treatment containing no antibacterial agents and the conception rate of 76 per cent for frozen semen both are comparable to the treatment and breeding results reported by Polge (9). The difference of 43 percentage units between the two treatments of the frozen semen has limited statistical importance, since there was no replication of semen samples and the number of services was small.

These data, and the unsatisfactory revival rates obtained when sulfanilamide was included in the extenders, suggest that any large scale experiment designed to test the effect of sulfanilamide on fertility of frozen semen would jeopardize the breeding efficiency of artificial insemination centers.

Toxicity of sulfanilamide in frozen extenders. The mean motility rate of spermatozoa in previously frozen extenders containing sulfanilamide was 33.0 per cent. By contrast, the mean motility rates of the spermatozoa in extenders containing no sulfanilamide were 53.1 per cent for the frozen extender and 51.2 per cent for the freshly prepared extender. The differences were highly significant ($P < 0.01$). These data indicate that sulfanilamide-containing yolk-citrate extenders, when frozen, become toxic to spermatozoa.

Glycerol was not used in these extenders since spermatozoa cannot be introduced directly into an environment of 10 per cent glycerol without injurious effects (1, 10). The data reported herein indicate that sulfanilamide should not be added to extenders which are to be frozen and stored for subsequent use as semen extenders. Satisfactory viability of spermatozoa in frozen semen extenders stored at -18 to $-22°$ C. with no antibacterial agents or with a combination of penicillin and streptomycin was reported by Hurst (3).

SUMMARY

The effects of two levels (presence and absence) of sulfanilamide, penicillin, and streptomycin on viability of spermatozoa stored at $-75°$ C. were studied in a 2^3 factorial experiment with semen from ten different bulls.

A reduction in viability of spermatozoa ranging from 29 to 43 per cent was attributed to the presence of 0.3 per cent sulfanilamide in samples stored at $-75°$ C. The treatment effect was highly significant ($P < 0.001$). Revival rates with all treatments ranged from 46 to 83 per cent. A low range of 46 to 50 per cent revival was obtained in samples with all treatment combinations containing sulfanilamide, the treatment effect being highly significant ($P < 0.001$).

Preliminary fertility data with frozen semen from one Holstein bull resulted in 60- to 90-day non-return rates of 76 per cent for frozen semen in the extender containing no antibacterial agents and 33 per cent for the extender containing sulfanilamide, penicillin, and streptomycin. Since semen from only one collection from one bull was used, the difference of 43 percentage units has limited statistical importance with respect to treatment effects on fertility of frozen semen.

A study of viability of spermatozoa stored 48 hours at 5° C. in extenders frozen and thawed prior to the addition of semen samples was made to test for toxic effects of sulfanilamide. The mean motility of spermatozoa from 16 ejaculates was 33.0 per cent in extenders containing sulfanilamide and 53.1 per cent in extenders containing no sulfanilamide. The difference of 20 percentage units was highly significant ($P < 0.01$).

The data reported herein suggest that sulfanilamide should not be included in yolk-citrate extenders to be frozen. A choice of antibiotics can be made only after fertility data have been obtained.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of E. O. Conrad and staff of the Wisconsin Scientific Breeding Institute and the cooperation of the technicians of Dane-Iowa, Rock, and Waukesha Breeders' Cooperatives in performing the inseminations.

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LIPID AND LIPOPROTEIN CONSTITUENTS OF EGG YOLK IN THE RESISTANCE AND STORAGE OF BULL SPERMATOZOA [1]

R. F. KAMPSCHMIDT,[2] D. T. MAYER [3] AND H. A. HERMAN

Animal Husbandry, Agricultural Chemistry, and Dairy Husbandry Departments
University of Missouri, Columbia

Since Phillips (16) and Phillips and Lardy (17) recommended egg yolk as a constituent of media for the dilution and storage of spermatozoa, evidence has accumulated which demonstrates that egg yolk is a necessary requirement for maximum survival and the maintenance of the fertilizing capacity of these cells. The complex nature of egg yolk, however, made it difficult to ascertain the specific factors responsible for the beneficial effects and the manner in which they were producing these effects.

One of the important functions of egg yolk in a diluting medium was described in a series of papers by: Lasley *et al.* (9), Easley *et al.* (5), Lasley and Bogart (11), Lasley and Mayer (12), and Bogart and Mayer (2). In these papers it was suggested that egg yolk is important in protecting the spermatozoa from adverse environmental conditions. Mayer and Lasley (14) were able to isolate from egg yolk a small fraction, soluble in phosphate buffer, which would protect a higher proportion of the spermatozoa from temperature shock than would whole egg yolk. Bogart and Mayer (2) stated that spermatozoa may manifest resistance to many other adverse environmental conditions in addition to that against temperature shock.

Additional effects of egg yolk on spermatozoa were described by Tosic and Walton (18, 19, 20), who found that the addition of egg yolk to bull semen caused an immediate increase in 0_2 uptake, followed by a decrease. The dialyzable portion of egg yolk was reported by these authors to contain a substance or substances which inhibited respiration, whereas the non-dialyzable portion contained substances which enhanced respiration and preserved the fertilization potentialities of spermatozoa. Lasley and Mayer (13) showed that egg yolk had no effect on the respiration rate of ejaculated ram spermatozoa, but markedly increased the respiration rate of epididymal spermatozoa of the ram.

In the present paper the influence of the non-dialyzable lipid and of the lipoprotein constituents of egg yolk upon the survival of bull spermatozoa during prolonged storage periods is described.

MATERIALS AND METHODS

Collection and dilution. Samples were collected biweekly over a two and one-half year period from bulls of the University of Missouri dairy herd. Twelve

Received for publication January 19, 1953.

[1] Missouri Agricultural Experiment Station, Journal Series No. 1347.

[2] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] This investigation was supported (in part) by a research grant from the National Institutes of Health, Public Health Service, and from Armour Research Laboratories, Chicago.

different bulls of proven fertility of the Holstein, Jersey, and Guernsey breeds were used.

The semen was collected with an artificial vagina, cooled immediately to 15 to 20° C., and held at this temperature until the experimental period. The time between collection and dilution varied from one-half to one hour.

The volume ratio of semen to diluter in all experiments was 1:10. The ratio of egg yolk to the mixture of buffer and sugar solutions in the diluter was 1:4. All of the ingredients were dissolved separately in glass-distilled water in concentrations resulting in solutions isosmotic with semen plasma. These solutions were pipetted into flasks containing egg yolk to give the desired quantities in the various storage media. When the various fractions isolated from egg yolk were substituted for egg yolk in the diluting media, the amount added corresponded to the quantity of that particular egg yolk constituent normally present in a diluting medium containing fresh egg yolk.

The vials of diluted semen were placed in a beaker of water and cooled slowly in the refrigerator to a storage temperature between 4 and 7° C.

Evaluation. The semen samples were warmed to 37° C. before making motility estimates. The motility was scored in thirds of a unit from 0 to 5. Zero represented no motility, and 5 the maximum.

The proportion of the spermatozoa surviving a storage interval or cold shock was determined by the staining technique developed by Lasley *et al.* (8) and later modified by Easley *et al.* (5) and Mayer *et al.* (15). The staining mixture was composed of 2 per cent fast green (FCF) and 0.8 per cent erythrosin B in 100 ml. of M/8 phosphate buffer. The slides were made immediately after dilution, after 60-, 120-, and 240-hour storage intervals, and after cold shock. The survival percentage was obtained by dividing the fraction of spermatozoa unstained at the end of a given storage period by the fraction found unstained immediately after dilution.

The survival percentage after cold shock was calculated in the manner described above. The spermatozoa in 0.5 ml. of semen were subjected to a temperature of 0° C. for 10 minutes in the cold shock technique. This technique was described and discussed by Lasley and Bogart (10) and Lasley and Mayer (12).

The pH of the samples was determined with the aid of a Beckman glass electrode pH meter.

Preparation of lipoprotein from egg yolk. The egg yolk lipoprotein, lipovitellin, was prepared according to the method described by Alderton and Fevold (1); another lipoprotein, lipovitellenin, was isolated by the method of Fevold and Lousten (6). However, most of the lipoprotein used in this investigation was prepared by a technique based on the two methods described below. This material probably contained a mixture of lipovitellin and lipovitellenin.

The yolks of ten eggs were separated from the whites and washed with water; any adhering egg white then was removed by rolling the yolks on clean filter paper. The yolk membranes were broken and the contents collected. Each batch, consisting of ten egg yolks, was stirred rapidly with 150 ml. of cold saturated NaCl solution. The yolk-NaCl emulsion was extracted four times with 300 ml.

of ethyl ether to remove ether-soluble lipids. After the ether extractions, the NaCl solution was added to 25 volumes of water. A heavy white precipitate formed. The precipitate was separated and washed with three 100-ml. portions of ether. The precipitated lipoprotein fraction was purified by redissolving it in 10-per cent NaCl solution and then reprecipitating it by pouring the NaCl solution into six volumes of water. The resulting white flocculent precipitate was separated by centrifugation and washed several times with distilled water. The weight of the dried lipoprotein from ten yolks was 10.4 g., or about 7 per cent of the weight of the original separated yolk contents.

Preparation of lecithins and cephalins from egg yolk. The lecithins and cephalins from egg yolk were prepared by a modification of a method described by Chargoff (3, 4). The yolk of each egg was heated at 65° C. for 30 minutes with 50 ml. of absolute alcohol. The alcohol was separated by filtration, a fresh 25-ml. portion was added, and the heating was continued for 15 minutes. The wet residue was extracted three times with 25-ml. portions of ethyl ether. The alcohol and ether filtrates were combined and evaporated under vacuum to remove the alcohol and ether. The residue was taken up in 100 ml. of ether, and anhydrous Na_2SO_4 and finely powdered anhydrous Na_2HPO_4 were added. The mixture was chilled overnight, filtered through bentonite, and again left in contact with Na_2HPO_4 for several hours. The etheral filtrate was evaporated, under vacuum, to dryness and the residue dissolved in ether. The solution was cleared in the angle centrifuge and concentrated. The addition of three volumes of absolute alcohol produced a slightly oily precipitate of cephalin fractions, which were washed with alcohol and acetone and purified by reprecipitation with acetone from ether. The concentrated alcoholic mother liquors yielded the lecithin fractions on the addition of four volumes of acetone.

EXPERIMENTAL RESULTS

Nature of the factor from egg yolk which protects the spermatozoa from adverse environmental conditions. Previous work, by Mayer and Lasley (14), had resulted in a rather impure fraction isolated from egg yolk which would protect spermatozoa from temperature shock. The protection from temperature shock, therefore, seemed to be the best criterion available for the selection of the components of egg yolk which are necessary in the storage of spermatozoa.

Residual effects of egg yolk in the protection of spermatozoa from cold shock. It was deemed desirable to determine whether or not the effects of egg yolk in protecting spermatozoa from cold shock would remain after removal of the egg yolk. In order to accomplish this, several aliquots of the same semen sample were compared so that the effects of centrifugation and cold shock could be determined in both the presence and absence of egg yolk. In eleven separate comparisons, removal by centrifugation of the egg yolk from the medium was found to remove all of the protection normally afforded by egg yolk to the spermatozoa. This suggests that the egg yolk was not permanently altering the spermatozoa and that the egg yolk factor must be present in order to protect the spermatozoa.

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Solubility of the egg yolk factor, in acetone, ether, alcohol, acid, and base. Egg yolk could be precipitated repeatedly with large volumes of acetone, yielding a light yellow precipitate. This precipitate, after drying, protected most of the spermatozoa from temperature shock. However, it was still inferior to whole egg yolk in this respect. Extracting the acetone precipitate with alcohol and ether by the method of Mayer and Lasley (14) did not result in an improved product in the majority of the trials. However, in two separate comparisons this product was superior to whole egg yolk in protecting the spermatozoa from temperature shock.

Separate portions of fresh acetone precipitate of egg yolk were stirred thoroughly with acid solutions, alkaline solutions, or water. Each of these solutions then was filtered by vacuum to yield an almost water-clear filtrate. The alkaline solution was found to contain the factor which protected the spermatozoa from cold shock. Neither the acid nor the water solution gave any protection in this test.

After dialysis, the ability of the alkaline extract to protect the spermatozoa from cold shock was maintained. A fraction precipitated from the alkaline extract with acid at a pH between 6.8 and 7 also was active.

Protection of the spermatozoa from cold shock by lipoprotein and its component parts. The factor responsible for the protection of the spermatozoa was shown to be soluble in dilute alkali, insoluble in water, and nondialyzable. An investigation of some of the protein complexes, therefore, appeared to be in order. Preliminary trials with lipovitellin and lipovitellenin from egg yolk prepared according to the methods of Alderton and Fevold (1) and Fevold and Lousten (6) gave excellent protection to the spermatozoa as measured by the cold shock technique. Lipoprotein prepared according to the procedure outlined in the section on *Materials and Methods* was checked thoroughly for its ability to protect the spermatozoa from temperature shock. A comparison of this fresh extract of lipoprotein in a citrate and a $NaHCO_3$-glucose solution (7) with whole egg yolk added to the same solution is shown in Table 1. Results obtained with

TABLE 1

Survival of the spermatozoa after cold shock (average of 17 trials)

Diluting medium			
Egg yolk plus citrate	Lipoprotein plus citrate	Lipoprotein plus $NaHCO_3$-glucose	Citrate alone
(%)	*(%)*	*(%)*	*(%)*
81.3	83.1	75.3	40.6

Standard error of any mean difference = 2.2

a mixture of one part $NaHCO_3$ solution to four parts of glucose solution alone and with whole egg yolk are not given, since they were almost identical with those obtained with the citrate solution. The analysis showed no significant difference in survival percentage ($P < 0.05$) between lipoprotein or egg yolk in a citrate solution. The lipoprotein in a mixture of sodium bicarbonate and glucose

solutions was significantly ($P < 0.05$) lower in protecting the spermatozoa from cold shock than the same lipoprotein in a citrate solution. However, all of the solutions containing either lipoprotein or whole egg yolk were significantly higher ($P < 0.01$) than the buffer solutions without egg yolk.

In earlier experiments in this laboratory, extracting an acetone precipitate of egg yolk with alcohol and ether in a Soxhlet apparatus gave variable results, some preparations exhibiting good activity, others often devoid of any activity. These results suggested that perhaps the hot alcohol was splitting the active material into inactive components. An attempt was made to answer this question by splitting the lipoprotein according to the method of Chargoff (4). The lipoprotein linkage was broken by heating for 30 minutes at 65° C. with absolute ethyl alcohol, then centrifuged while warm, and the supernatant alcohol was removed. The alcohol extract contained the factor which protected the spermatozoa from temperature shock. The alcohol insoluble residue did not give any protection to the spermatozoa.

Since the hot alcohol extract of the lipoprotein would contain predominantly phospholipids, these were isolated from egg yolk by the methods described previously in this paper. The protection afforded the spermatozoa against temperature shock by egg yolk lecithin and cephalin prepared by these procedures is shown in Table 2. There was no significant difference ($P < 0.05$) between the egg yolk-

TABLE 2

Survival of spermatozoa after cold shock with egg yolk lecithin or egg yolk cephalin (average of 18 trials)

Diluting medium			
Egg yolk plus citrate	Egg yolk plus lecithin plus citrate	Egg yolk plus cephalin plus citrate	Citrate alone
(%)	*(%)*	*(%)*	*(%)*
75.1	74.3	72.7	45.3

Standard error of any mean difference = 2.2

lecithin or egg yolk-cephalin mixtures and whole egg yolk in their abilities to prevent the harmful effects of cold shock. The egg yolk lecithin or egg yolk cephalin was significantly higher ($P < 0.01$) than the medium without egg yolk.

Two commercial preparations of lecithin from soy beans were checked and found to protect the spermatozoa from cold shock equally as well as egg yolk.

Livability of the spermatozoa during storage in various fractions of egg yolk. If the only function of egg yolk in the diluting medium for spermatozoa were to protect against rapid drops in temperature, many of the fractions mentioned in the previous section should be effective during storage.

Lipoprotein, as previously described, was compared with whole egg yolk during prolonged storage. The lipoprotein either was used immediately after preparation or it was stored under water in a refrigerator prior to use. Both citrate buffer and a mixture composed of isotonic $NaHCO_3$ and glucose solutions were used with the egg yolk and the lipoprotein. Table 3 gives the average storage

TABLE 3

Livability of spermatozoa during storage with lipoprotein and whole egg yolk (average of 34 trials)

	Egg yolk-citrate solution	Egg yolk-(1 $NaHCO_3$ + 4 glucose) solution	Lipoprotein-citrate solution	Lipoprotein-(1 $NaHCO_3$ + 4 glucose) solution
60 hr. motility	1.92	2.57	1.13	2.55
120 hr. motility	0.95	2.37	0.22	2.37
240 hr. motility	0.43	2.12	0.00	2.11
Per cent survival (240 hours)	67.3	83.2	62.9	83.1

results of spermatozoa at 60, 120, and 240 hours for 34 comparisons in these diluters. In the sodium bicarbonate-glucose medium lipoprotein proved just as satisfactory as whole egg yolk for the storage of bovine spermatozoa. With citrate buffer, the lipoprotein was inferior to whole egg yolk.

Since the intact lipoprotein was effective in the storage of spermatozoa at low temperatures, the alcohol extract and the alcohol-insoluble residue of lipoprotein were tried. The results with citrate buffer will not be shown, since they were consistently lower with all of the extracts.

It will be noted from Table 4 that neither the alcohol extract, the residue, nor a combination of the two was as effective as the entire intact lipoprotein during prolonged storage.

TABLE 4

Livability of spermatozoa during storage with some of the component parts of lipoprotein in a (1 $NaHCO_3$ + 4 glucose) solution (average of 14 trials)

	Lipoprotein	Alcohol extract of lipoprotein	Residue from alcohol extraction of lipoprotein	Combined extract and residue
60 hr.	2.76	2.02	1.70	1.22
120 hr.	2.57	1.67	0.92	0.97
240 hr.	2.28	1.18	0.26	0.55

Numerous trials were conducted also with lecithin and cephalin since they were very effective in protecting the spermatozoa from cold shock. However, both of these phospholipids gave poor results in the prolonged low temperature storage of spermatozoa.

It is quite obvious that something is needed besides these phospholipid fractions which protect the spermatozoa from cold shock. The additional storage value of lipoprotein might be provided by the protein part of the molecule. Since the protein part of the egg yolk lipoprotein is a phosphoprotein resembling casein, attempts were made to add casein to the medium. The spermatozoa were stored in diluters prepared by various methods and composed of lecithin or cephalin and casein. Results with these media in no way approached those obtained with lipoprotein. Various amino acids such as glycine, cystine, and

tryptophan, as well as globulin and albumin proteins from blood, gave equally disappointing results.

All previous reference to lipoprotein, as used in this investigation, has been to the fresh material extracted from egg yolk or to this material after storage under water in a refrigerator at 3° C. It was noted early in these investigations that drying, either in air or a vacuum desiccator, decreased the solubility of the lipoprotein. When the solubility was decreased, it no longer gave satisfactory protection against rapid drops in temperature or provided satisfactory conditions for the storage of spermatozoa. Various methods of drying were tried in an attempt to prevent the resulting decrease in activity. Among the methods used were lyophilization, the repeated precipitation of the lipoprotein with acetone and then drying in air, or a similar method using ether instead of acetone. The third method resulted in greatly decreased solubility and poor activity of the lipoprotein. With both the lyophilization and acetone drying, even though the solubility was diminished, the ability to protect against temperature shock remained, but at a slightly lower level than with whole egg yolk. Table 5 presents

TABLE 5

Comparisons of the livability of spermatozoa in media with lipoprotein dried by acetone or lyophilization with fresh lipoprotein, in a sodium bicarbonate-glucose solution (average of 19 trials)

	Fresh lipoprotein	Acetone dried lipoprotein	Lyophilized lipoprotein
60 hr.	2.41	2.18	2.47
120 hr.	2.28	1.96	2.35
240 hr.	2.06	1.11	1.40

a comparison of the storage results with the dried lipoprotein and the fresh material. The dried materials provided satisfactory storage conditions for the spermatozoa during the first five days, but after this time a rapid decline in the motility rating occurred.

DISCUSSION

Perhaps the most important problem confronting investigators in the field of artificial insemination is that which is primarily concerned with the various aspects of the storage of mammalian spermatozoa. Storage encompasses two categories of spermatozoan physiology, viz., survival and maintenance of the fertilizing capabilities of these cells. It is generally assumed that spermatozoan survival and maintenance of the fertilization potentialities are related phenomena and that any change in the environment of these cells during storage which is beneficial to one would benefit the other.

In the past it has been difficult to test this hypothesis because our knowledge of spermatozoa physiology and chemistry has been limited and the storage medium in which both survival and fertilizing capacity were best maintained contained the biologically and chemically complex mixture of substances comprising egg yolk. It is possible that results of this preliminary investigation

which suggest that a lipoprotein from egg yolk can satisfactorily replace the complex egg yolk in storage media may be a first step toward the elucidation of the problem of the maintenance of survival and the fertilizing capabilities of these atypical cells.

Lipoproteins are complexes about which we know comparatively little, since their chemistry and their role in the physiological activities of the cell have not been thoroughly investigated. Recent research suggests that lipoproteins are of physiological importance to other types of cells besides mammalian spermatozoa.

The most salient feature of egg yolk as a constituent of diluting media up to the present time seemed to be its unique ability to protect spermatozoa from adverse environmental conditions, such as temperature shock. However, in line with previous observations by Bogart and Mayer (2), egg yolk apparently contributes two distinct factors, one of which protects these cells against cold shock (a resistance factor) and a second which contributes in some manner to the maintenance of spermatozoan survival (a storage factor). Interestingly, as shown by the present investigation, phospholipids from egg yolk act as a resistance factor, but not as a storage factor; whereas the lipoprotein complexes containing a phospholipid act both as a resistance factor and as a storage factor.

The results presented in Tables 3 and 4 indicate: (a) that something in addition to the protection from temperature shock is required by the spermatozoa during prolonged storage and (b) that the requirements for satisfactory storage of spermatozoa are provided by the lipoprotein complexes prepared by accepted methods. However, it cannot be stated with certainty whether the protein in the complex or the entire complex is acting as the storage factor. The lowered storage value of a medium containing the lipid and protein portions of the complex may be due to one of several factors. The intact lipoprotein may be required during storage, and the splitting of the lipid-protein linkage destroys the activity. It is also possible that the protein alone may possess activity, but the hot alcohol treatment used in splitting the complex may have denatured the protein or otherwise affected its activity.

At present the authors are unable to present any definite theory regarding the mode of action of either of these non-dializable factors.

SUMMARY

The lipid portion of a lipid-protein complex which occurs in egg yolk, and which can be extracted in rather pure form, will protect the spermatozoa from temperature shock. This lipid portion consists mainly of the phospholipids, lecithin, and cephalin, and these obtained from a variety of sources were effective in protecting bull spermatozoa from cold shock. The protection from temperature shock, in the presence of phospholipids, is more satisfactory in citrate or phosphate solutions than it is in solutions of non-electrolytes such as glucose.

The results of this investigation show that other substances, in addition to those required for the protection of the spermatozoa from temperature shock, are needed during the prolonged storage of bull spermatozoa at low temperatures.

The lipoproteins from egg yolk serve as satisfactory substitutes for egg yolk in the storage of bovine spermatozoa. However, these lipoproteins must be used fresh or must be dried by methods which will not markedly decrease their solubility in order to obtain maximum survival during the storage of bovine spermatozoa.

ACKNOWLEDGMENT

The authors are indebted to Armour Research Laboratories, Chicago, for the cephalin used in these experiments.

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ANTIBIOTICS IN RUMEN DIGESTION AND SYNTHESIS
III. THE EFFECT OF AUREOMYCIN ON RUMEN MICROORGANISMS, WITH SPECIAL REFERENCE TO THE STREPTOCOCCI AND COLIFORM GROUPS [1, 2]

C. M. CHANCE,[3, 4] C. K. SMITH, C. F. HUFFMAN, AND C. W. DUNCAN
Departments of Dairy, Bacteriology, and Agricultural Chemistry, Michigan Agricultural Experiment Station, East Lansing

The use of some antibiotics and feed supplements containing antibiotics to stimulate growth in certain farm mammals has been established, but the mode of action of these selective or inhibitory chemical agents is relatively unknown insofar as their effect on the microorganisms in the bovine rumen is concerned.

Bartley *et al.* (2) could not find a consistent difference between rumen microorganisms in control and aureomycin-fed calves by using a direct microscopic method. Neumann *et al.* (22) observed that the total bacterial counts in the rumen ingesta of aureomycin-fed heifers were about the same as in control heifers, although the types of microorganisms were less diverse when aureomycin was fed. This observation suggested that aureomycin can inhibit some of the naturally-occurring rumen flora. In an *in vitro* study of cellulose digestion with rumen microorganisms, Wasserman *et al.* (31) found that low concentrations of penicillin and streptomycin stimulated the cellulolytic microorganisms and neomycin was stimulatory in all concentrations used, whereas chloromycetin inhibited the cellulolytic microorganisms. A corresponding increase in total bacterial count was observed also as cellulolytic activity increased.

Other investigators have found that the composition of the ration has a definite influence on the number, size, and types of rumen microorganisms (1, 4, 5, 8, 9, 10, 11, 12, 19, 24, 25, 26). A high grain ration has been shown to decrease the microorganisms which are characteristically associated with a relatively high roughage ration (24, 25, 26). Burroughs *et al.* (10) suggested that the mineral constituents in roughage make an important contribution to the metabolism of the cellulolytic microorganisms. Bentley *et al.* (4) indicated that the low phosphorus content of poor quality hay was a limiting factor in cellulose digestion. The total bacterial count was decreased markedly when starch was added to a roughage ration, but the count increased when casein

Received for publication January 19, 1953.

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal article No. 1443.

[2] This study was made in part with funds provided by the Research and Marketing Act of 1946, through a cooperative project between the Michigan Agricultural Experiment Station and the Bureau of Dairy Industry.

[3] This article is part of a dissertation presented to the Faculty of the Graduate School of Michigan State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[4] Present address: Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

replaced the starch (9). This finding lends support to the theory that proteins aid in roughage digestion by furnishing essential nutrients to the cellulolytic microorganisms.

The numbers of bacteria found by various investigators in the rumen of cattle have been reviewed by Gall *et al.* (15), and a criteria for distinguishing true rumen microorganisms has been reported by Gall and Huhtanen (16).

Because of the lack of information in the literature concerning the effect of antibiotics on rumen microorganisms in the intact bovine, an investigation was made to determine the influence of aureomycin on the pH and the total bacterial counts in rumen ingesta and feces, with special reference to the streptococci and coliform groups. These groups were selected only because they represent the two large groups of organisms classified according to the Gram staining technique and would probably indicate a characteristic response to the antibiotic. For purposes of comparison, similar determinations were made on the same steers maintained on the identical unsupplemented ration.

EXPERIMENTAL

Data concerning two rumen-fistulated steers, the composition of the ration, and the collection of samples have been reported previously (13). A ration consisting of 4 lb. of corn and 15 lb. of alfalfa-brome hay was fed once daily. Samples of rumen fluid were collected for bacterial and pH determinations at 2-hour intervals for 12 hours on each of the first 3 days for both levels of aureomycin intake. The rumen fluid was withdrawn from the rumen with a sterile pipette connected to a large rubber bulb. At each collection period, a pH determination was made with a Beckman pH meter, and a 5-ml. sample of rumen fluid was preserved for total bacterial count in 10 ml. of a 10-per cent formalin solution. Cultural studies for the presence of streptococci and coliform microorganisms were made on another portion of fresh rumen fluid collected at each 2-hour interval. The rumen fluid was transported in a screw-cap tube filled to capacity to maintain anaerobic conditions.

Bacterial studies were made on the feces from the steers for both levels of aureomycin intake. The fecal samples were collected once per day (before feeding), three times per week throughout the period for each level of aureomycin intake. The fecal material was collected manually from the steer and placed in a sterile petri dish. Immediately after collection, a 10-g. sample was added to 90 ml. of buffered distilled water in a sterile flask, made up to 100 ml., and thoroughly dispersed. A 10-ml. portion was preserved in 5 ml. of 40-per cent formalin solution for total bacterial count. The remaining portion was used for cultural determinations for the presence of streptococci and coliform microorganisms.

The microscopic procedure developed by Bortree *et al.* (6) was used for making the total bacterial counts. For the cultural studies, 1 ml. of the fresh rumen fluid was placed in 99 ml. of sterile buffered distilled water to yield a 1:100 dilution. For the fecal samples, 1 ml. of the original 1:10 dilution was added to a sterile 99-ml. blank to yield a 1:1,000 dilution. A 1:100 dilution also was

made by adding 10 ml. of the original 1:10 dilution to a sterile 90-ml. blank. For both the rumen and fecal samples, the remaining dilutions necessary for the analyses were made from the above dilutions. Lauryl sulfate tryptose broth, prepared according to the formula of Mallmann and Darby (17), was used to determine the coliform microorganisms, and dextrose azide broth (18) was used to determine the streptococci in rumen and fecal material. The reliability of these methods for use with rumen contents had been determined previously. The highest dilution in which turbidity could be observed was used to determine the number of rumen or fecal streptococci present. The highest dilution showing gas formation was used to indicate the number of coliform organisms.

RESULTS AND DISCUSSION

Figure 1 shows graphically the pH and the total bacterial counts of the rumen contents from steers fed 4 lb. of corn and 15 lb. of alfalfa-brome hay daily with and without aureomycin. The data represent the average of that obtained at 2-hour intervals for 12 hours on the first 3 days of each period. The pH of the rumen contents from steer 714 for each level of aureomycin intake was consistently lower than that observed for steer 707 during the same period. The pH was lower when the control ration was fed than when the ration was supplemented with aureomycin. The pH decreased in both steers after feeding and reached the lowest point in about 6 or 8 hours, followed by a slight increase about 10 hours after feeding. This fact is not entirely in agreement with the observations of several investigators (20, 21, 30) who reported that the pH declined for about 4 hours and then increased gradually after that time. A possible explanation for the difference observed in this investigation may be the fact that the steers were fed only once daily, whereas the animals in the

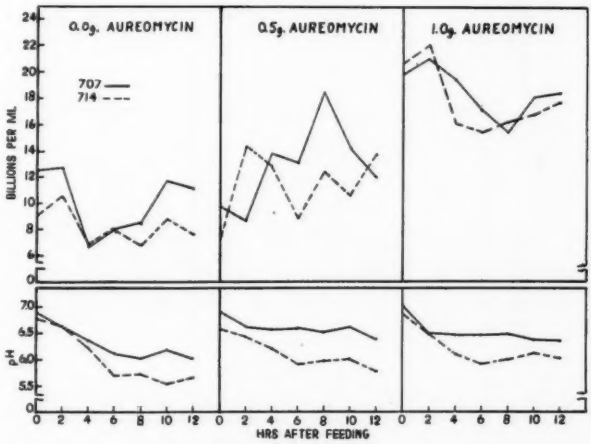

FIG. 1. pH and total bacterial counts of the rumen contents obtained from steers fed aureomycin (average of the first 3 days of each 15-day period).

other experiments were fed twice daily. Roine and Elvehjem (28) stated that the pH was not determined by the kind of food but by the kind of microflora which developed in the digestive tract. Gall *et al.* (15) and Phillipson (23) observed that the type of ration has some effect on the pH of the rumen contents of cattle and sheep.

The bacteriological data presented in Figure 1 show a definite increase in the total bacterial count in the rumen contents when 0.5 g. of aureomycin was added to the ration and a further increase in total count when the aureomycin was increased to 1.0 g. per day. The additional increase may have been due to a stimulatory effect on some particular rumen microorganisms. Data published earlier (13) showed that dry matter, crude protein, crude fiber, and nitrogen-free extract were removed from the rumen about 20 per cent faster when 0.5 g. of aureomycin was included in the ration than when omitted from the ration. The increased removal was attributed to a stimulatory effect on the cellulolytic bacteria (13), since more bacteria were present to digest the feed. On the other hand, the decrease observed in the removal of these nutrients when 1.0 g. of aureomycin was fed may have been due to an inhibitory action on the cellulolytic bacteria and a stimulatory effect on the noncellulolytic microorganisms. In any case, the symbiotic relationship between the rumen microorganisms was disturbed. The bacterial counts made periodically throughout each of the 15-day trials remained approximately at the same level as those observed for the first 3 days. The total counts obtained in this investigation are lower than those reported by Gall *et al.* (15), which were made on rumen liquor rather than on

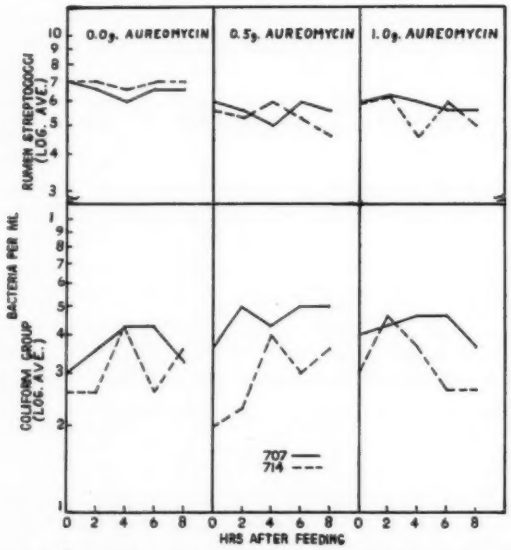

FIG. 2. Influence of aureomycin on the streptococci and coliform groups in the rumen of steers (average of the first 3 days of each 15-day period).

the natural rumen contents, but they are in close agreement with the counts reported by Bryant and Burkey (7).

There was a reduction in the number of rumen streptococci for both steers when aureomycin was included in the ration (Figure 2). In the coliform group, the number observed in steer 707 was consistently higher than that found in steer 714. The increase in the coliform group when 0.5 g. of aureomycin was fed may be of some significance because it was at this concentration that the highest removal of the various nutrients from the rumen was observed (13). It is interesting to note that the rate of removal of the various nutrients from the rumen of steer 714 was lower than that observed in steer 707 and that this coincided with a lower number of coliform organisms and total bacterial count. The over-all rate of removal of the nutrients from the rumen was higher in both steers when 0.5 g. of aureomycin was fed, although the highest percentage of removal occurred in steer 707 (13).

Bartley *et al.* (2) and Rusoff (29) did not observe any consistent difference in the rumen microflora between the control and aureomycin-fed calves. Even though the total counts were about the same for both the control and the aureomycin-fed heifers, Neumann *et al.* (22) noted that the types of bacteria in the heifers receiving aureomycin were much less diverse, which suggested that the

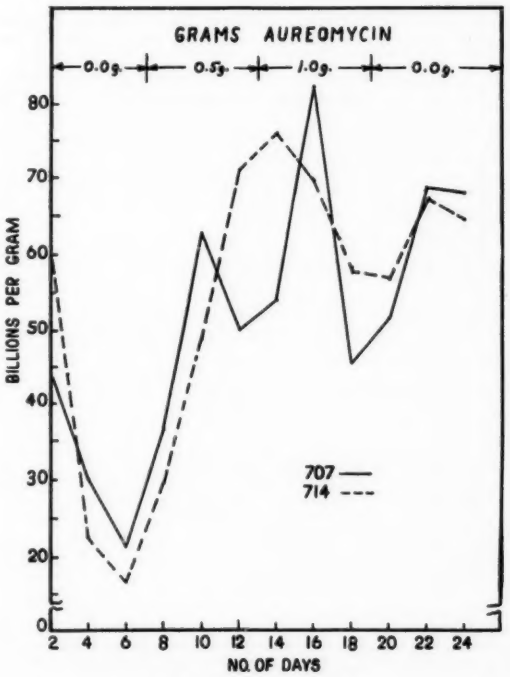

FIG. 3. Total bacterial counts of feces obtained from steers fed aureomycin.

normal rumen flora had been disturbed. The ration that Neumann *et al.* fed consisted of a high proportion of grain, and some reduction in appetite was observed when aureomycin was added to the ration. Bell *et al.* (3) reported more severe results, but they also fed a ration high in grain and fed a higher concentration of aureomycin than Neumann *et al.* It is possible that the rumen flora adapted to a high proportion of grain may be sensitive to aureomycin and that the flora which developed on the normal hay and grain ration fed in the present study could withstand a higher concentration of aureomycin. In the present study, the amount of aureomycin fed daily was much higher than that fed by either Bell *et al.* or Neumann *et al.*

Figure 3 shows the bacterial total counts of the fecal material obtained from steers fed aureomycin. Results of the cultural studies for the streptococci and the coliform groups in the feces are shown in Figure 4. These data indicate an increase in the total bacterial count in the fecal material when aureomycin was fed. This may have been caused by the growth-stimulating effect of the antibiotic or a depressing effect on certain organisms, thereby allowing other organisms to predominate and attain a number higher than the previous total count. The decrease in the total bacterial count prior to the feeding of aureomycin cannot be explained, but if the highest count in the control period is used as a standard, an increase in the total bacterial count is still in evidence.

There was very little difference between the number of streptococci in the

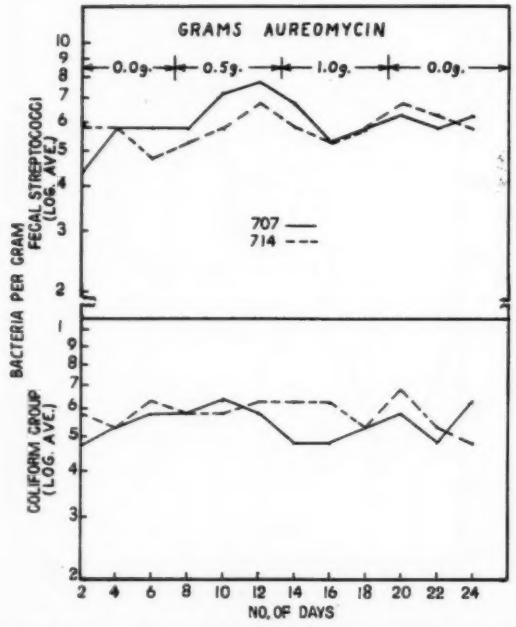

FIG. 4. Influence of aureomycin on the streptococci and coliform groups in the feces.

rumen and fecal material, but the number of coliform microorganisms in the feces was higher than that observed in the rumen ingesta. The fact that there was no discernible effect on the numbers of streptococci or coliform groups when the aureomycin was fed was surprising at first. The 0.5 g. level of aureomycin increased the numbers of fecal streptococci slightly, but with 1.0 g. the number dropped to the control level and remained relatively constant. Radisson (27) has shown that some of the organisms of these two groups have the ability to adapt themselves quickly to various concentrations of antibiotics. The actual number of organisms remains approximately the same, but the metabolism appears to be altered. This could explain, in part, the relative constancy of the numbers of these groups during all phases of this work.

SUMMARY

Two rumen-fistulated steers were used to study the effect of aureomycin on the total bacterial counts and the streptococci and coliform groups in rumen ingesta and feces.

The pH of the rumen content declined for about 6 to 8 hours after feeding, with only a slight increase at the end of 10 hours. When aureomycin was included in the ration, the pH was not as low as when the aureomycin-free ration was fed; however, the response was in the same direction.

A definite increase occurred in the total bacterial count of the rumen contents and feces when aureomycin was included in the ration. The number of rumen streptococci decreased, whereas the number of coliform organisms remained approximately the same in one steer and increased in the other. There was a direct correlation between the increase in the coliform population in the rumen when 0.5 g. of aureomycin was fed and the increased removal of dry matter, crude fiber, crude protein, and nitrogen-free extract from the rumen.

ACKNOWLEDGMENT

The aureomycin used in these experiments was supplied by Lederle Laboratories Division, American Cyanamid Co. The authors also thank Mrs. Carol L. Frank for conducting the bacteriological analyses.

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BAKING PROPERTIES OF FROZEN CONDENSED SKIMMILK

L. V. ROGERS, R. W. BELL, AND T. J. MUCHA

Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture, Washington, D. C.

That the casein complex of concentrated skimmilk slowly undergoes denaturation during frozen storage has been recognized for some time (4). Denaturation, which is manifested by the appearance of flakes and is followed by separation of insoluble curdy particles in the thawed product, is retarded by storage at low temperatures (1) and the addition of cane sugar (2).

Frozen condensed skimmilk, when properly frozen and thawed, was found to be a satisfactory source of serum solids for ice cream (3). However, little attention has been given to the utility of this thawed skimmilk in the making of bread, where milk solids are known to be beneficial not only from a nutritive standpoint but also because of improvements in color, texture, and shape of the finished loaf. If thawed condensed skimmilk containing the casein complex in its denatured as well as its natural state should prove to be a satisfactory source of nonfat milk solids in bread, then the value of frozen storage as a means of preserving these solids would be greatly enhanced. Thus, a company with milk concentrating equipment and refrigeration facilities, but no dryer, would be able to preserve surplus skimmilk for baking, as well as for ice cream making and possibly other uses, from the period of maximum milk production to the time when there is a shortage of this commodity. It was with this in mind that the following study of the baking properties of frozen condensed skimmilk was made.

METHODS AND MATERIALS

The skimmilk was divided into two equal portions, one of which was given a heat treatment of 82.2° C. for 30 minutes, which was considered as the "high-heat treated" milk. The other portion was held at 62.8° C. for the same time and was considered the "low-heat treated" milk. At the end of the holding period the skimmilk was cooled to 48.8° C., drawn into a condensing pan, and concentrated under 27 in. of vacuum to 27 per cent solids, or to approximately three times its original solids content. After this step was completed, each lot of condensed skimmilk was divided into two portions; one portion was canned as such, and to the other 10 per cent by weight of granular sucrose was added prior to canning in No. 3 cans. After being sealed, the cans were placed in frozen storage at −17° C., with duplicate samples stored at −22° C. Samples were removed periodically over approximately a year and thawed in a water bath that was maintained at 21.1° C. After a thawing period, the cans of milk were opened, their contents were poured into a suitable container, and the physical appearance of the product was noted. After moderate mixing, a portion was reconstituted to 9 per cent total solids and used in determining the solubility index expressed

Received for publication January 22, 1953.

as milliliters of sediment per 50 ml. of sample. A 50-ml. portion was placed in a graduated conical tube and centrifuged at 1,000 r.p.m. for 5 minutes. One-half (0.5) ml. was considered the maximum sediment in stable milks. The reconstituted skimmilk was used to evaluate flavor.

In the baking procedure the concentrated skimmilks were reconstituted to their original solids level and the proper amount was added to the bread doughs. Two loaves were baked for each variable and a repeat baking was made on the following day, or a total of four loaves for each sample of thawed skimmilk.

A 60-per cent, 3-hour sponge procedure with the following formula was used in making the white pan bread:

Ingredients	*Parts*
Flour	100.00
Sugar	5.00
Salt	2.00
Lard	2.00
Malted wheat	0.50
Yeast food	0.25
Yeast	2.00
Mold inhibitor	0.10
Fluid skimmilk	68.50
Water	8.70

The flour was a mixture of equal parts northwestern and southwestern baker's patent, and one lot of flour was used for the duration of the experiment. The optimum absorption for this mixture was 65 parts water per 100 parts flour. Originally it was intended to utilize only fluid skimmilk (9 per cent milk solids and 91 per cent water) or 71.5 parts as the liquid for making the dough. This amount would contain 6.4 parts of milk solids and would correspond to more than the amount necessary for 6 per cent milk solids bread. In order to adjust the milk solids level more nearly to 6 per cent, only 68.5 parts of reconstituted skimmilk, which contained 6.2 parts of solids and 62.3 of water, were used. An additional 2.7 parts of water were added to the dough to meet the required flour absorption and, in order to allow the solids of the fluid skimmilk an equal weight of water, as is customary when dried skimmilk solids are used, six parts of water were added to each dough. Thus a total of 8.7 parts of water was added to each dough in addition to the reconstituted skimmilk.

Proper allowance was made in the dough formula for the sugar content of the samples which contained added sugar.

All doughs were mixed in a laboratory size vertical mixer; turning the doughs was done by hand; molding, panning, and baking were carried out mechanically. The ingredients other than the skimmilk were commercial grade, and all steps in the bread-making procedure followed accepted, commercial practice.

As the experiment progressed, the physical condition of the frozen condensed skimmilk deteriorated, and, in some instances, the thawed samples appeared

totally unsuited for bread-making purposes. However, the curd in even the most precipitated samples could be dispersed satisfactorily by ½ minute mixing in a Waring blendor; the samples then could be diluted with water and readily incorporated with the other dough ingredients.

RESULTS AND DISCUSSION

Table 1 presents the calculated volume per cent, based on the control for each loaf volume average obtained over a period of 371 days of the four loaves that were baked on two successive days at the 6 per cent milk solids level and contained the reconstituted frozen condensed skimmilk.

TABLE 1

Per cent of control loaf volume for bread made with frozen condensed skimmilk

Storage time of frozen condensed skimmilk	Volume of control loaf, using dried nonfat milk solids	Low-heat treated skimmilk (62.8° C. for 30 min.)				High-heat treated skimmilk (82.2° C. for 30 min.)			
		With added sugar—stored at		Without sugar—stored at		With added sugar—stored at		Without sugar—stored at	
		−17° C.	−22° C.	−17° C.	−22° C.	−17° C.	−22° C.	−17° C.	−22° C.
(Days)	*(Ml.)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
7	2494	95.2	92.7	93.2	92.5	96.7	99.0	101.4	98.2
14	2475	93.6	96.0	97.0	96.5	100.0	100.5	98.0	95.2
21	2525	93.5	93.5	96.3	92.1	97.5	98.8	99.0	96.3
60	2562	99.1	100.7	100.0	91.5	102.5	98.8	100.7	95.4
120	2550	94.1	94.1	92.6	94.9	102.4	96.8	92.4	97.1
180	2500	96.5	95.5	94.7	89.5	96.2	99.0	91.2	92.0
371	2113[a]	97.6	99.7	97.9	99.7	106.8	106.4	99.1	104.1

[a] Low volume for control probably is due to decrease in baking quality of the flour after 1-yr. storage.

The loaf volume obtained with the dried skimmilk was the control for judging the baking quality of the frozen skimmilk as reflected in the loaf volume obtained at the various time intervals.

There was no substantial decrease in the baking quality of the frozen condensed skimmilk under any of the various conditions of storage. With each set of storage conditions, with few exceptions, the samples which received the higher heat treatment produced larger loaf volumes. This relationship held over the period of the experiment and is in accordance with the known beneficial effect of proper heat treatment of milk used for baking. In the examination of both the low- and the high-heat treated samples, no advantage was found in storing the frozen samples at −22° C. over storage at −17° C., since neither storage temperature produced samples which consistently showed a larger loaf volume compared with the control. In a comparison of the volumes obtained when cane sugar in the thawed condensed skimmilk was the only variable, it will be seen that the sucrose had no consistent effect on the loaf volume.

Table 2 presents the effect of the treatment and storage temperature on the characteristics of the thawed samples before they were used in the baking of the bread. The samples were removed, thawed, and examined after 2, 7, 14, 21, 60,

120, 180, and 371 days of frozen storage. After 2, 7, and 14 days of storage, no significant changes were observed, except that at the 14-day period the samples with added sugar were slightly wheaty (malty or raw cereal-like) and those with no added sugar were slightly flat (lacked flavor); the low-heat treated samples

TABLE 2

Effect of treatment and storage at various temperatures, with and without added sugar, on the properties of the condensed skimmilk used in the baking tests of Table 1

Heat treatment	Sugar added	Storage temperature	Storage time	Appearance of thawed milk [a]	Sediment	Flavor
(° C.)	*(%)*	*(° C.)*	*(days)*		*(ml.)*	
62.8 for 30 min.	0	−17	21	Mod. gel-like dep.	0.1	Normal
	10	−17		Mod. gel-like dep.	0.1	Normal
	0	−22		Mod. gel-like dep.	0.2	Normal
	10	−22		Sl. gel-like dep.	0.2	Normal
82.2 for 30 min.	0	−17		Lumpy dep.	0.6	Normal
	10	−17		Sl. gel-like dep.	0.1	Normal
	0	−22		Mod. lumpy dep.	0.1	Normal
	10	−22		Sl. gel-like dep.	0.1	Normal
62.8 for 30 min.	0	−17	60	Abund. gel-like dep.	5.0	Flat
	10	−17		Abund. farina-like dep.	0.3	Sl. ox.
	0	−22		Gel-like, lumpy dep.	0.2	Flat
	10	−22		Sl. rice-like dep.	0.1	Flat
82.2 for 30 min.	0	−17		Abund. farina-like dep.	11.0	Tr. acid
	10	−17		Abund. farina-like dep.	0.2	Flat
	0	−22		Sl. dep., sl. curdy	1.0	Tr. acid
	10	−22		Sl. dep., v. fine	0.1	Normal
62.8 for 30 min.	0	−17	180	Curdy with whey	11.0	Sl. wheaty
	10	−17		Abund. cryst., sl. curdy	0.6	Ox. (not typical)
	0	−22		Sl. gel-like, sl. curdy	4.0	Ox., wheaty
	10	−22		Mod. curdy and cryst.	0.3	Sl. ox., wheaty
82.2 for 30 min.	0	−17		Abund. curdy with whey	13.0	Tainted
	10	−17		Abund. curdy, cryst. dep.	2.0	Sl. heated
	0	−22		Abund. farina-like dep.	11.0	Flat, sl. heated
	10	−22		Mod. curdy, cryst. dep.	0.3	Flat
62.8 for 30 min.	0	−17	371	Curdy with whey	11.0	Flat, wheaty
	10	−17		Sl. gel-like dep.	0.5	Ox., wheaty
	0	−22		Tr. gel-like dep.	3.0	Flat, tr. acid
	10	−22		Sl. gel-like dep.	0.3	Wheaty
82.2 for 30 min.	0	−17		Curdy with whey	.....	Flat
	10	−17		Buttermilk consistency	2.0	Flat
	0	−22		Buttermilk consistency	0.3	Tr. acid
	10	−22		Sl. curdy dep.	0.2	Flat

[a] Tr. = trace; sl. = slight, slightly; mod. = moderate; st. = strongly; cryst. = crystalline; ox. = oxidized; gel. = gelatinous; dep. = deposit; abund. = abundant.

contained only traces of sediment, whereas the high-heat treated samples had a measurable amount (0.1 ml.) whether they contained added sugar or not. The results of the 120-day examination were not included in Table 2 because they were essentially the same as those observed at the 180-day period. Although slight flavor changes took place within the first 14 days of storage and body deterioration became evident in a full set of samples in 21 days, no thawed skim-

milk, when incorporated in doughs, produced breads of objectionable odor, flavor, or appearance at any baking trial throughout the 371 days of the experiment. The fact that an acceptable loaf of bread can be made from doughs that contain 6 per cent milk solids-not-fat derived from thawed, destabilized, and reconstituted skimmilk, of even deteriorated flavor, should be of considerable interest and potential importance to the dairy industry.

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INFLUENCE OF RATE AND TEMPERATURE OF COOKING ON THE TIME REQUIRED TO MANUFACTURE CHEDDAR CHEESE FROM PASTEURIZED MILK

W. W. OVERCAST, E. R. JARMAN,[1] AND T. W. ALBRECHT
Tennessee Agricultural Experiment Station, Knoxville

The influence of temperature on the rate of acid production by cheese cultures has been studied by several investigators in this country and abroad. Whitehead and Cox (8) found that certain strains of streptococci organisms in cheese cultures developed acid normally during the early stages of cooking and up to a temperature of 98.6° F. but produced acid slowly during the remainder of the cheese manufacturing process. Golding, Amundson, and Wagenaar (4) found that a temperature of 102° F. retarded acid development of commercial lactic starters. In the cheese making operation Elliker (3) suggests a maximum temperature of 102° F., to be reached within 45 minutes and the curd held at this temperature for 1 to 1.5 hours. Price (7) also recommends a cooking temperature of 102° F. and that the final temperature be reached in 30 minutes. He suggests raising the temperature at the rate of 1° F. in the first 5 minutes, 2° F. each 5 minutes for the next 10 minutes, then 3° F. every 5 minutes until the final temperature of 102° F. is reached. Wilster (10) uses a slower rate to raise the temperature, suggesting an increase of 1° F. for each 5 minutes for the first 15 minutes, then increasing the rate until a temperature of 98 to 101° F. is reached within 35 to 40 minutes. Also, he states that only on rare occasions should the cooking temperature be below 98° F. or above 102° F.

Whitehead and Hunter (9) showed that cheese cooked at 100° F. reached an acidity of 0.50 per cent 40 to 60 minutes before cheese which was cooked at 104° F. With the higher cooking temperature, acid development was definitely retarded in the latter stages of the operation. Babel (2) also showed the importance of temperature on acid development in both cultures and cheese making. He studied seven cultures at 86, 98, 101, and 104° F. Of these cultures, three produced acid most rapidly at 86° F., and four at 98° F. All cultures produced acid more slowly at temperatures of 101 and 104° F. In actual cheese-making operations a cooking temperature of 102° F. slightly retarded acid production of five of the seven cultures when compared with a cooking temperature of 100° F. All of these cultures were retarded appreciably when a cooking temperature of 104° F. was used.

Abd-El-Malek and Gibson (1) made a detailed study of the chemical and physical characteristics of the streptococci of milk. They concluded that *Streptococcus lactis* could grow and reproduce at 104° F. but that *Streptococcus cremoris* could not grow at that temperature. Nichols and Hoyle (6) studied 277 strains of streptococci isolated from commercial starters and 72 strains suitable for

Received for publication January 22, 1953.

[1] Present address: Georgia Mountain Experiment Station, Blairsville, Ga.

cheese making isolated from sour milk. All the starter strains examined were *S. cremoris* and those (with two exceptions) from sour milk were *S. lactis*. They concluded that *S. cremoris* is the lactic acid-producing organism most often found in commercial cheese starters.

EXPERIMENTAL PROCEDURE

The milk used for making the experimental cheese was Grade A for the local market. The milk was pasteurized by heating to 143.5 to 144° F. and holding for 30 minutes. Immediately after cooling, the milk was placed in the cheese vats. Three of these were 50-gal. vats and the fourth was a 100-gal. vat. Four vats of cheese were made at each trial. Two were made according to the cooking procedure herein described and two by the method outlined by Price (7). Twenty-four vats of cheese were made, using six different cultures.

The milk was tempered to 86° F., and 1 per cent lactic starter was added. The milk and inoculum were mixed thoroughly and ripened for 45 minutes. Rennet was added at the rate of 3 oz. per 1,000 lb. of milk, and the milk was stirred for 7 minutes. Coagulation occurred in 12 to 13 minutes, and the curd was cut with ¼-in. curd knives 30 minutes after adding the rennet. The curd was allowed to set about 5 minutes, after which it was stirred slowly.

Heating of the curd began 15 minutes after cutting. In each trial two vats were heated according to Price's schedule (7). In the other two vats heat was applied at a rate to raise the temperature 1° F. each 5 minutes. The final temperature of 98° F. was reached 1 hour after the steam was turned on. Special care was taken to maintain the same rate of cooking on all duplicates. The steam was turned off and stirring was continued for an additional 15 minutes after reaching the maximum temperature of 98° F. The curd was allowed to settle for another 15 minutes before draining. Two hours and 15 minutes after the addition of the rennet, the whey was drained. At this time the acidity was determined. Draining required less than 5 minutes, and the curd was packed and cut into slabs about 8 in. wide. The slabs were turned at about 10-minute intervals. The titratable acidity was checked about every 10 minutes, and when it reached 0.25 per cent the slabs were piled two high, and when it reached 0.30 per cent the slabs were piled three high. The slabs were turned and repiled every 10 minutes until ready to mill. The cheese was milled at 0.40 per cent acidity. The curd was stirred after milling to prevent matting, and within 15 minutes salt was added in three portions at the rate of 2.5 lb. per 1,000 lb. of milk. The curd was stirred after each addition of salt. The curd was placed in Young American type hoops 15 minutes after salting. The hoops were placed in the press for 30 minutes at 16 lb. pressure. The pressure then was released and the cheese was dressed, after which it was returned to the press for 16-18 hours. The cheese was allowed to dry for 3 to 4 days at 50° F. It then was paraffined and cured at 50° F. All lots were analyzed for fat, moisture, total nitrogen, and water soluble nitrogen. The cheese was scored for flavor by four impartial judges.

RESULTS

A comparison of the two methods of manufacture is shown in Table 1. The rate of acid development from draining to milling in the curd cooked at 98° F. was approximately twice as fast as that of the curd cooked at 102° F. The average time from draining to milling when using the slow heat-low heat method was 65 minutes as compared to 125 minutes when using the method outlined by Price (7). The average time from adding rennet to milling was 3 hours and 20 minutes for the slow heat-low heat method and 4 hours and 20 minutes for Price's method.

TABLE 1

A comparison of the slow heat-low heat method and Price's method for the manufacture of cheddar cheese from pasteurized milk

Operation	Slow heat-low heat method			Price's method		
	Time	Temp.	Acid	Time	Temp.	Acid
		(°F)	(%)		(°F)	(%)
Pasteurization	7:30	144	0.18	7:30	144	0.18
Added starter	8:40	86	0.18	8:40	86	0.18
Added rennet	9:25	86	.....	9:25	86	.....
Coagulation	9:37	86	.....	9:37	86	.....
Cutting	9:55	86	0.12	9:55	86	0.12
Steam on	10:10	86	.....	10:10	86	.....
Steam off	11:10	98	.....	10:40	102	.....
Settling	11:25	98	.....	11:25	102	.....
Draining	11:40	98	0.16	11:40	102	0.14
Packing	11:45	98	0.18	11:45	102	0.15
Piling 2 high	12:05	97	0.25	12:35	101	0.25
Piling 3 high	12:15	96	0.30	1:00	99	0.30
Milling	12:45	96	0.40	1:45	97	0.40
Salting	1:00	95	.....	2:00	96	.....
Pressing	1:15	....	.....	2:15	....	.....
Dressing	1:45	....	.....	2:45	....	.....

The rate of acid development of each of the six cultures at cooking temperatures of 98° and 102° F. is presented in Table 2. All six cultures produced acid more rapidly at 98° F. than at 102° F. Acid production of culture 712 was retarded less at 102° F. than it was in other cultures. This was expected, since culture 712 was a single strain of *S. lactis*, which, according to Abd-El-Malek and Gibson (1), grows at the higher temperature. The various cultures showed

TABLE 2

Time required from the addition of rennet to milling to develop a whey acidity of 0.40 per cent at cooking temperatures of 98° and 102° F.

Culture	98° F.		102° F.		Difference in minutes
	Hours	Minutes	Hours	Minutes	
F D	3	25	4	20	55
H P_1	4	35	5	32	57
146	3	17	4	05	48
712	3	10	3	35	25
T T	3	05	4	07	62
H P_2	3	15	4	20	65

variations in the length of time required to develop a whey acidity of 0.40 per cent from 5 minutes to 1 hour and 30 minutes at 98° F. and up to 1 hour and 57 minutes at 102° F.

The average time required for all six cultures to reach a milling acidity of 0.40 per cent when the slow heat-low heat method was used was 65 minutes as compared to 125 minutes for the Price method. In all trials the titratable acidity of the whey was two to four points higher at the time of draining when the slow heat-low heat method was used.

A panel of four judges found no appreciable difference in the flavor scores of the cheese made by the two methods. One lot using *S. lactis* 712 was bitter. Since the cheeses manufactured by both methods using culture 712 were bitter it appears that this defect was due to the culture used rather than the method employed.

DISCUSSION AND SUMMARY

The data presented indicate that the rate and temperature of cooking Cheddar cheese made from pasteurized milk materially influence the time required for the manufacturing operation. The rate of cooking and the final temperature reached in cooking govern the acid production of the culture organims. Commercial cheese cultures are generally various strains of *S. lactis* and *S. cremoris* or mixtures of these plus the associated organisms. Nichols and Hoyle (6) found *S. cremoris* to be the predominating organisms in cheese cultures used in England. It seems reasonable to believe that this would be the case also in the United States.

There was no appreciable difference in the flavor scores of the cheese made by the two methods. However, Lockry *et al.* (5) felt that too rapid a rate of acid development during the making process produced cheese of high acid and bitterness. This increased rate of acid production by the slow heat-low heat method did not produce these defects.

The cheese made by the slow heat-low heat method developed from 0.02 to 0.04 per cent more acidity during the cooking process than that made by the Price method. This would indicate that the organisms were able to produce acid during the cooking period, whereas with Price's schedule this acid development did not occur, indicating that temperatures employed inhibited detectable acid production. With the Price schedule the temperature was above the growth range of the organisms within 25 minutes after cooking began. Under these conditions *S. cremoris* produces little acid, and therefore the rate of heating is probably just as important as the final temperature reached in cooking.

The difference in time required to manufacture the cheese was about 60 minutes in favor of the slow heat-low heat method. This saving in time was true in all trials, except where a pure strain *S. lactis* 712 culture was used. This culture produced acid more rapidly at 98° F. than at 102° F., but this difference was not as great as when mixed multiple strain cultures were used.

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BLOCK VS. LOOSE SALT CONSUMPTION BY DAIRY CATTLE

S. E. SMITH, F. W. LENGEMANN, AND J. T. REID
Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

The literature on livestock feeding is replete with miscellaneous observations on the voluntary intake of salt by dairy cattle and other farm animals. Rarely does one find the results of well-designed studies of salt consumption and the variables that may affect it. In fact, the authors know of none with dairy cattle. Among the many possible variables is the physical form of salt offered, that is, loose, rock, or block salt. Of these, loose and block salt are the most commonly used, although reliable figures as to the relative amounts of each are not available. Block salt has become popular because it is manufactured to a high state of hardness to withstand leaching. How the cow reacts to this is largely a matter of speculation. The extreme point of view is that a cow cannot or will not get her needed salt requirements from this hard block. To test this view, the following studies were conducted, which, in addition, furnished information on the effect of some feeds (types of pasture) on the salt intake.

EXPERIMENTAL PROCEDURE

A factorial design involving 24 lactating cows (18 Holsteins and six Brown Swiss), three types of pastures, and two forms of common feeding salt was used as follows:

Pasture Type	*Form of salt*	*Replicates*
1. Kentucky bluegrass-white clover	Block	4
	Loose	4
2. Alfalfa-Ladino clover-Brome grass	Block	4
	Loose	4
3. Ladino clover-orchard grass	Block	4
	Loose	4

The treatment groups of four cows each were balanced as to the stage and level of milk production and breeds. In addition to pasture, the cows were fed a simple concentrate mixture of corn, oats, wheat bran, soybean oil meal, and dicalcium phosphate. The daily concentrate allotment varied from time to time, depending on the quantity of pasture herbage, but for any given time all cows received the same amount. In 1949 and in 1951 the concentrate mixture contained 1 per cent salt, in keeping with common feeding practice. In 1950 the concentrate mixture contained no supplemental salt. Thus in 1949 and 1951, the cows received supplemental salt from the concentrate mixture and in addition either block or loose salt according to plan. In 1950 the only source of supple-

Received for publication January 31, 1953.

mental salt was either the block or loose salt provided on pasture. Once cows were allotted to either block or loose salt treatment they continued on the same treatment for the duration of the pasture season.

The salt consumption was recorded at weekly intervals during the pasture seasons for each group of four cows. The salt provided was dried to constant weight and the weighbacks again were dried before weighing. While on pasture the salt was so protected by covered boxes that no leaching losses occurred.

For an analysis of the collected data, the weekly intakes of salt by each group of four cows were calculated to a basis of salt intake per cow per day. These data then were reduced for interpretation by analysis of variance.

RESULTS

A summary of the gross data for the three summer seasons is presented in Table 1. Each datum is the average salt intake per cow per day, calculated from the weekly consumption of each group of four cows. Each period is of a week's

TABLE 1

Loose vs. block salt consumption of dairy cows

Period	1949		1950		1951	
	Loose salt	Block salt	Loose salt	Block salt	Loose salt	Block salt
	Kentucky bluegrass-white clover					
	(g.)	*(g.)*	*(g.)*	*(g.)*	*(g.)*	*(g.)*
1	24	6	195	70	63	26
2	26	85	114	61	62	1
3	28	12	112	8	77	20
4	94	98	141	27	132	14
5	....	....	89	43	35	15
6	....	....	138	45	9	10
7	....	....	52	29	17	2
8	....	....	.....	....	37	11
9	....	....	.....	....	50	2
	Alfalfa-Ladino-Brome pastures					
1	13	26	99	61	13	38
2	1	15	35	24	143	6
3	8	24	79	43	179	27
4	15	5	15	39	156	28
5	....	....	89	38	49	7
6	....	....	99	22	128	6
7	....	....	76	46	44	14
8	....	....	....	....	152	18
9	....	....	....	....	152	5
	Ladino-orchard pastures					
1	41	10	90	77	13	43
2	12	6	57	41	88	1
3	35	12	24	15	163	30
4	64	2	17	6	30	30
5	....	....	35	58	19	12
6	....	....	83	47	97	1
7	....	....	25	41	27	15
8	....	....	....	....	131	36
9	....	....	....	....	110	5
Av.	30	25	79	40	81	15

duration. It should be kept in mind also that the cows in 1949 and 1951 received, in addition, salt from the concentrate mixture. Those in 1950 depended entirely for supplemental salt on either the loose or block salt.

The analysis of variance for each year of observation indicated the following: In 1949 there were no significant differences between amounts of salt consumed from loose or block forms; among amounts of salt consumed on the three pasture types, nor among periods of observation. In 1950 the differences between loose and block salt consumption was significant (1 per cent level). Here the cows consumed almost twice as much loose as block salt. There were also highly significant differences in the salt consumed among the pasture types, being highest on Kentucky bluegrass-white clover. The salt intake × pastures interaction was likewise highly significant. The more extensive observations in 1951 did not agree with the comparable conditions of 1949. In 1951 the difference between loose and block salt intakes was significant at the 1 per cent level. The consumption of loose salt exceeded that of the block salt some five times. Differences in salt intakes on the different pastures as well as among the periods were significant at 5 per cent levels.

It is obvious that in 2 out of the 3 years, the cows voluntarily consumed considerably more loose than block salt, whereas during the first year there was no significant difference. It should be pointed out that the data of the first year were more limited than in the other two. There is also strong evidence of a difference in salt consumption on different types of pasture. The basis of this variation was not ascertained.

An obvious question is, "Did the cows fed block salt get enough to satisfy their requirements?" An analysis of the milk production records showed no significant difference in the production levels of the two groups, from which it may be assumed that the consumption of the block salt met dietary needs. Also, data from studies of salt requirements yet to be published denote that even the lower levels of salt consumed were adequate for lactation needs. Apparently then, the salt consumed as loose salt in excess of that consumed as block salt was luxury consumption.

TABLE 2

Loose vs. block salt intakes of dairy heifers on pasture

	I			II			III			IV			V		
	Number	Days	Av. daily intake	Number	Days	Av. daily intake	Number	Days	Av. daily intake	Number	Days	Av. daily intake	Number	Days	Av. daily intake
			(g.)			*(g.)*			*(g.)*			*(g.)*			*(g.)*
Heifers (14-24 mo.)	30	14	Block 1 Loose 22	30	10	Block 1 Loose 35	21	6	Block 2 Loose 22						
Heifers (2-3 yr.)	38	10	Block 2 Loose 46	33	18	Block 1 Loose 46	33	7	Block 4 Loose 21	35	9	Block 1 Loose 21	61	6	Block 1 Loose 22

Grand av. for block salt—2 g.; loose salt—29 g.

As a secondary study, salt intakes of heifers on pastures were measured under conditions where both loose and block salt were available. For these animals improved pastures of several types constituted the only feed. A summary of the data is found in Table 2. Each salt consumption datum is the average per heifer per day calculated from each period of observation.

Thus these heifers which had a choice of both forms of salt consumed decidedly more loose than block salt.

A general perusal of these data indicates great variability in salt consumption even under fairly uniform environmental conditions. Such variation undoubtedly would be larger if the salt intake had been measured for each animal each day. This suggests conservatism in interpreting salt consumption data. It also serves as a challenge to factor out some of the major causes affecting salt intakes.

SUMMARY

In 2 out of 3 years, lactating cows on pasture voluntarily consumed significantly more loose than block salt. However, the lower intakes of salt as block salt were adequate to meet the needs for lactation. The intake of salt was significantly influenced by the type of pasture furnished the cows.

ACKNOWLEDGMENTS

The authors acknowledge the aid of Professor C. R. Henderson in analyzing the data and the Salt Producers Association for financial assistance.

STUDIES OF HEATED MILK. V. THE REACTION OF LACTOSE WITH MILK PROTEIN AS SHOWN BY LACTOSE -1- C^{14} [1]

STUART PATTON AND ROBERT J. FLIPSE
The Pennsylvania Agricultural Experiment Station, State College

If the many mechanisms of lactose destruction which appear to operate in heated milk are to be understood, some technique of following the phenomena with precision must be developed. As yet there is no method with sufficient specificity by which total lactose destruction in heated milk can be measured. Larsen and Gould (5) have demonstrated this in a recent study comparing results of three conventional methods for measuring lactose in milk. One method, as yet unadapted to milk, is that refined by Horowitz *et al.* (3) and originally reported by Fearon (2). It depends upon a colorimetric reaction of lactose with methylamine and NaOH. This reaction should be highly specific for lactose in milk. Glucose, galactose, fructose, sucrose, starch, and many sugar degradation products do not interfere (2).

Even if total lactose destruction could be determined accurately, the problem of what happens to the lactose would remain. In an effort to clarify this problem, studies with the aid of lactose–1–C^{14} as a tracer compound have been initiated. The present paper deals with a quantitative study of lactose-protein interaction as a result of heat treatment. Subsequent research will attempt to elucidate the mechanisms of formation of certain lactose decomposition products through study of labeled carbon distribution.

EXPERIMENTAL

Lactose–1–C^{14}, specific activity approximately 1 microcurie per milligram, was obtained from the National Bureau of Standards. One microcurie of the labeled lactose was used per 25-ml. sample of fresh, mixed herd, raw whole milk. During heating, the milk samples containing the isotope were retained in 40-ml. Pyrex centrifuge bottles. These bottles were plugged with rubber stoppers tightly held in place by wiring to the necks of the bottles. In order to check the temperature of the milk, one sample in each series was fitted with a thermometer through the rubber stopper. Heat treatment was effected in an oil or water bath by means of an electric hot plate or bunsen burner, depending on the temperature desired. Preliminary cooling was accomplished by placing the samples under cold running tap water; final cooling, in ice water. One-inch Visking tubular membrane was used to dialyze uncombined lactose and other milk constituents from the protein. Dialysis was carried out in the cold (4° C.) with daily changes of distilled water. The end-point of dialysis was determined with the aid of Molisch's test performed by mixing two drops of reagent with

Received for publication February 9, 1953.

[1] Authorized for publication Feb. 4, 1953 as paper No. 1783 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

1 ml. of diffusate and underlaying with approximately 2 ml. of concentrated H_2SO_4. The absence of any purple color at the interphase or in the mixture after shaking the tube was interpreted as a negative test, and dialysis was considered complete at this point. This required a period of 3 to 4 days, during which time the samples within the membrane nearly doubled in volume. The dialyzed samples, as well as an unheated, undialyzed control, were adjusted with distilled water to 50 ml. One-ml. quantities were placed in 1⅛-in. aluminum planchets and then evaporated to dryness with an infra-red lamp. Radioactivity of the dried preparations was determined by a windowless flow gas G.M. counter in conjunction with a decade scaling unit. A series of planchets prepared from the same sample by this procedure indicated variability due to sample preparation of less than 3 per cent. Mean counts per minute were obtained by averaging the results of six 2-minute counting cycles after correcting for background and unheated, dialyzed sample values. The per cent lactose combined with the protein was determined by attributing a value of 100 per cent to the radioactivity of the unheated, undialyzed control. The expression of activity as per cent lactose is reasonably correct, in all probability; however, it must be remembered that some quantity of decomposition products containing the isotope also may have been bound to the protein. Determinations of the degree of "browning" were made on 25-ml. portions of the heated, dialyzed milk samples according to the method of Choi *et al.* (1) as applied to heated, fluid milk systems by Patton (10). Fearon's test for lactose was employed in the manner described by Horowitz *et al.* (3).

RESULTS AND DISCUSSION

Formation of a lactose-protein complex. In order to determine the magnitude of activity bound by the protein which could be expected on heating to various temperatures, 25-ml. samples of milk containing 1 microcurie of lactose–1–C^{14} were heated to temperatures of 60°, 105°, and 135° C. momentarily, and immediately cooled. Only the protein from the 135° C. sample demonstrated any reaction with lactose, and this was equivalent to 0.8 per cent of the lactose originally present in the milk. No discoloration was evident as a result of the heat treatment afforded these samples.

Results of this preliminary trial indicated that the point in question could be studied more effectively by using holding heat treatments. A series of samples was heated at 97 ± 1° C. for time intervals up to 60 minutes. The time to reach temperature in this experiment was 11 minutes. Results regarding "browning" and lactose binding by the milk protein are presented in Figure 1A. Similar experiments were conducted with holding temperatures of 115 ± 2° and 121 ± 3° C. These data are presented in Figures 1B and 1C, respectively. The time to reach temperature with the 115° samples was 16 minutes; with the 121° samples, 26 minutes. The results of these experiments show that color formation closely parallels combination of lactose with the milk protein. In addition, they indicate that the binding reaction commences prior to color development.

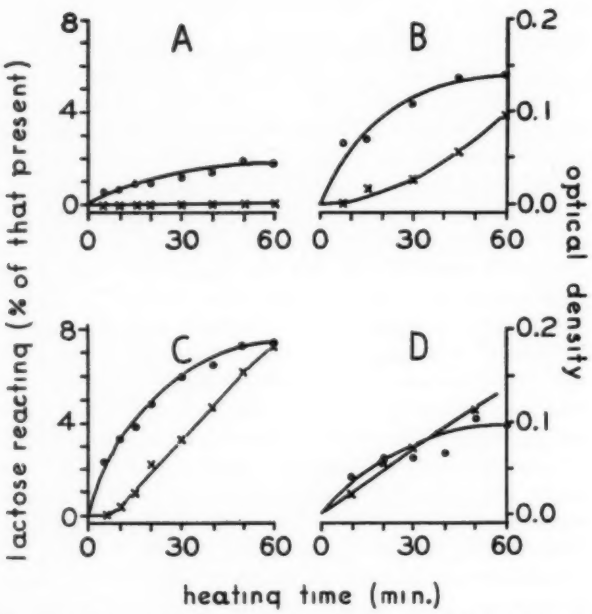

FIG. 1. Reaction of lactose with milk protein (O——O) and color development (X——X) in heated milk systems:—Milk heated at 97 ± 1° C. (A), 115 ± 2° C. (B), 121 ± 3° C. (C) and a lactose-casein system at 115 ± 2° C. (D).

This is best illustrated at 97° C. but is plainly evident also in the 115° and 121° C. experiments.

An additional trial was conducted to determine whether a system composed only of lactose and casein would yield results materially different from those obtained with whole milk. A further purpose of this experiment was to obtain information regarding the reaction ratio of lactose and casein under various conditions of heat treatment. The casein sol was prepared by supercentrifuging raw skimmilk at 30,000 r.p.m. for 30 minutes, resuspending the protein deposited in the bowl in distilled water, and dialyzing the suspension to remove other milk constituents. The sol was corrected to the original volume of skimmilk employed. Added to the sol were ordinary lactose to a concentration of 5 per cent and radioactive lactose at the rate of 1 microcurie per 25 ml. of sample. The casein content of the lactose-casein system was determined to be 2.34 per cent by Kjeldahl analysis for nitrogen. Data concerning "browning" and radioactivity associated with the protein following various heat treatments of the system are presented in Figure 1D. A laboratory accident prevented determination of color in the 40- and 60-minute samples. With the exception that color formation commences with somewhat less rigorous heat treatment in the simplified system, the lactose-casein solution appeared to behave very similarly to milk. The difference regarding color development may have resulted from the in-

hibiting action of sulfhydryl compounds formed during heating of the milk. The lower level of lactose binding in the simplified system as compared with milk heated at 115° C. probably resulted from the difference in protein concentration of the two systems. Assuming a value of 55.1 mg. of free amino nitrogen per gram of total protein nitrogen (Lea and Hannan, 6), it can be calculated that much less than a quantitative reaction between lactose and the amino groups of casein occurred. Such a reaction ratio would require binding of approximately 10 per cent of the lactose originally present in the system. One reason why this may not be attained became clear in subsequent experiments regarding the fate of lactose bound to the milk protein.

Stability of lactose-protein complex. Milk, containing added lactose–1–C^{14}, was heated at 97° C. for 1 hour and the lactose-protein complex thus formed was recovered by exhaustive dialysis. Radioactivity associated with the protein was determined. Ordinary lactose was added to 25-ml. quantities of the dialyzed material in amounts to yield 5 per cent solutions of the sugar. These samples were autoclaved for various times at 122° C. A control sample with no lactose added was included and received maximum heat treatment (60 minutes). The samples were cooled immediately and again exhaustively dialyzed to recover the protein suspensions which were plated in a manner to correct for volume changes during dialysis and then counted. The data, Table 1, show that the lactose-protein complex is labile to heat, as evidenced by the loss of approximately two-thirds of the radioactivity originally associated with the protein. The control sample heated for 60 minutes demonstrates that this is true whether the complex is heated in the presence or absence of lactose. A further point of interest concerning this sample is that it underwent a slight but definitely noticeable degree of browning with only the lactose bound to the protein present. Destruction of the complex apparently does not involve the regeneration of lactose. Fearon's test for lactose was negative both before and after autoclaving the complex at 122° C. for 1 hour. As employed at this laboratory, this test is sensitive to as little as 0.1 mg. of lactose. It was determined that the

TABLE 1

The effect of heat treatment [a] *on the stability and color of a lactose-1-C^{14}-protein complex* [b] *in a 5 per cent lactose solution*

Holding time	Activity [c]	Optical density
(min.)	*(c.p.m.)*	
Control (no heat)	90 ± 3 [d]	0.000
10	45 ± 1	0.030
20	31 ± 2	0.064
30	28 ± 1	0.132
40	29 ± 3	0.154
50	31 ± 1	0.238
60	30 ± 2	0.288
60[e]	28 ± 2	0.015

[a] Autoclaved at 122° C. for the indicated holding times.

[b] Prepared by heating milk, with lactose-1-C^{14} added, at 97° C. for 1 hr. followed by exhaustive dialysis to recover the aqueous complex which then was made 5% with ordinary lactose.

[c] With standard error indicated.

[d] Equivalent to binding of 2.25% of the lactose originally present in the milk.

[e] No lactose added.

solutions tested could contain as much as 1.1 mg. of lactose if all the lactose bound by the protein were to react in the test. These findings indicate that the combination of lactose with milk protein is primarily a chemical reaction, rather than a physical adsorption. The precise chemical nature of the lactose-protein complex and its heat decomposition products is not known; however, the Molisch test revealed that some form of carbohydrate is involved. Proteins recovered from heated (97° C.-1 hour) milk by isoelectric precipitation, dialysis, or supercentrifugation all have been observed to give a strong Molisch test and a negative Fearon test. The intensity of the Molisch test on protein from heated milk is much greater than the faint reaction obtained with protein from unheated milk.

The foregoing results concerning the interaction of lactose and protein in heated milk suggest two important chemical mechanisms. The first involves the formation of a relatively stable end-product. This mechanism accounted for the binding of approximately one-third of the lactose reacting with milk protein at 97° C. during 1 hour. The second concerns a more dynamic mechanism in which lactose appears to form an unstable complex with the protein. With the continuous formation and destruction of such a complex, very substantial quantities of lactose could undergo decomposition. In such a mechanism milk protein may be regarded as a catalyst in lactose destruction; indeed, this conception seems to be in keeping with the available facts. Although pure lactose solutions are relatively stable to heat, the presence of casein in such solutions promotes the formation of numerous lactose decomposition products which have been conclusively identified (4, 7, 8, 9, 11) and brings about the discoloration commonly known as "browning." It is not clear at this point how the above mentioned mechanisms are involved in browning; however, the question should yield to further experimentation.

The results reported here are somewhat in contrast to those obtained by Lea and Hannan (6) working with protein-reducing sugar systems in the "dry" state. Their work has shown that the reaction between glucose and the free amino groups of casein can be carried nearly to completion. In the present study with fluid milk subjected to rigorous heat treatment, the reaction appeared to be less quantitative and the end product much less stable. Such a variation in findings might be expected in view of the differences in conditions of reaction employed in the two investigations.

SUMMARY

The reaction of lactose with protein in heated milk was studied with the aid of lactose–1–C^{14}. The amount of radioactivity bound by the protein is positively related to the extent of heat treatment and the degree of "browning." In heated milk, binding of lactose by the protein is initiated prior to color development. A substantial part of the lactose, protein complex appears to be unstable to heat. The significance of the unstable portion of the complex to the destruction of lactose in heated milk is discussed.

ACKNOWLEDGMENT

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 423 in the series of papers approved for publication. The views or conclusions contained in the report are those of the authors and are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

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THE EFFECT OF RELAXIN AND MECHANICAL DILATION ON THE BOVINE CERVIX[1]

EDMUND F. GRAHAM[2] AND ARTHUR E. DRACY
South Dakota Agricultural Experiment Station, Brookings

If the transfer of ova is to become practical, simple non-surgical techniques must be employed. One of the greatest obstacles encountered in nonsurgical techniques for obtaining ova is the difficulty of dilating the cervix at the appropriate time to allow the use of instruments in the uterus. To facilitate entrance into the uterus, some mechanical or hormonal means of dilating the cervix is desirable. Experiments on small laboratory animals suggest the possibility of using relaxin, which has the profound effect of relaxing the symphisis pubis (1, 2, 4, 5, 7) and may relax the cervical muscle. However, according to Hisaw (6) the animal must be under the influence of the follicular hormone before relaxin can produce symphyseal relaxation. At that time, Hisaw did not know that corpus luteum produced both progesterone and relaxin. Later work by Albert, Money, and Zarrow (3) showed that other parts of the ovary also contain relaxin. Also, Frieden and Hisaw (5) demonstrated some very important physiological actions of relaxin upon connective tissue.

In order to obtain information as to the possibility of dilating the cervix, a series of experiments was devised to answer the following questions:

1. Does the administration of relaxin in conjunction with the estrogen, diethylstilbesterol, serve to relax the cervical muscle?
2. What is the minimum dosage of relaxin which may be effective? the optimum? Is estrogen alone sufficient to relax the cervix?
3. Can a mechanical dilator be made to dilate the cervix?
4. Is the process of mechanical or hormonal administration accompanied by harmful effects to the tissue of the cervix or uterus?

EXPERIMENTAL AND RESULTS

Eight cows were used in this investigation. Three cows were assigned to an experimental group, three to a control group, and two were used for a pilot investigation.

To determine the minimum amount of relaxin to use on the experimental cows, a series of injections was given to a pilot cow. She was given injections of relaxin with an increasing quantity at each successive treatment. She was sensitized with an estrogen 3 days in succession prior to each of the relaxin treatments.

Received for publication February 17, 1953.

[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station as Journal Series No. 282.

[2] These data are taken from a thesis presented to the graduate school of South Dakota State College by the senior author in partial fulfillment of the requirement for the Master of Science degree.

This was done by giving diethylstilbesterol at the rate of 20 mg. per day. Cervical dilation was estimated by manual palpation of the cervix as constricted, intermediate, or relaxed. This was determined by placing the hand in the vagina and estimating the cervical relaxation by entering the cervix with the finger. If the finger could not be forced into the external os, the cervix was considered constricted. If the finger could go only past the first cervical ring, the cervix was considered intermediate. If the finger could go past the second ring, the cervix was considered relaxed. There was no response to the injection of 25 or 50 G.P.U. [3] of relaxin; the cervix was considered constricted on examination following each dosage. A small response was noted following the injection of 100 G.P.U. of relaxin, and the cervical tonus was considered as intermediate. An injection of 250 G.P.U. of relaxin appeared to produce a definite response, and the cervical tonus was considered as relaxed. These injections were given at a time when the cow was not in estrus. The cervix was palpated before the beginning of each series of injections and was in all cases found to be constricted. The cervix was palpated also just prior to the injection of relaxin and was found to be constricted. From these data a dosage of 250 G.P.U. of relaxin was chosen as a minimum for the experiment.

A second pilot cow was used to determine the effect upon the cervix of injecting 20 mg. of diethylstilbesterol 3 days in succession with no relaxin. No relaxation was palpable following this treatment.

As relaxin relaxes the cervical muscles, some means of actually dilating the cervix in conjunction with the relaxed muscles is desirable. Therefore a mechanical instrument was made to open the cervix so that some type of ova collector could be inserted into the cervix.

Five different instruments were designed for this purpose. Each of these instruments was tested on animals not assigned to this experiment so that the cows on the experiment could be treated in the same manner with the instrument best suited.

The instrument found to be best suited was made of carefully machined bronze. The body of the instrument consisted of 1¼-in. tubing, 6 in. long. Four tool steel prongs were inserted into the head of the tubing. The prongs, which were ¼-in. wide and 8 in. long, were bent from the head to meet 5 in. from the end. The ends of the rods were joined to make one smooth rod 5/16 in. in diameter. A plunger then was placed inside the bronze tubing with a wheel and screw on the posterior end to force the plunger against the inside of the four prongs, forcing them to open. The prongs were held tightly together by a rubber band. This precision-made instrument (Figure 1) was satisfactory.

Three cows were assigned to an experimental group to receive relaxin, and three were kept as controls. Cervical tonus was estimated by palpation during estrus, 5 days post estrus, and 10 days post estrus. The cervical dilator was used at estrus and 5 days post estrus to test the effects of relaxin injections upon the muscle of the cervix. Five days post estrus was selected as the day to give

[3] One Guinea Pig Unit of relaxin is the minimum quantity required to relax the pubic symphyses of approximately two-thirds of the guinea pigs in a group of twenty.

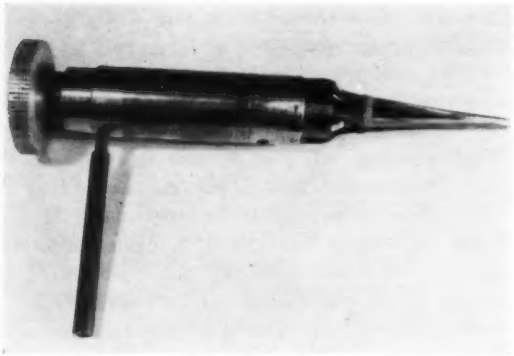

FIG. 1.

the cows the relaxin because normally the fertilized ovum enters the uterine horn about 72 hours post estrus. However, after 5 days post estrus it is necessary to open the cervix for the collection of ova.

Relaxin was given to the same three cows for three consecutive estrous cycles. The relaxin was injected subcutaneously 12 hours before the cervix was to be dilated. Three different dosages of relaxin were given. Five days post estrus of the first, second, and third estrous cycles 250, 1,500, and 8,500 G.P.U., respectively, were given.

During the three estrous cycles, a certain degree of relaxation was observable in all six animals as measured by manual palpation. The cervical relaxation was estimated as intermediate or relaxed. Five days post estrus of each cycle it was noted that the experimental cows differed markedly from the untreated control cows in the degree of palpable relaxation of the cervix. As shown by Table 1, the animals sensitized 3 days in succession with 20 mg. of diethystilbesterol and injected with relaxin indicated more relaxation than did untreated cows. The degree of relaxation varied with the amount of relaxin given. Table 1 also indicates that 1,500 G.P.U. of relaxin gave more cervical relaxation than 250 G.P.U. but that 8,500 G.P.U. gave but little more relaxation than did 1,500 G.P.U. Palpating the cervix 10 days post estrus showed that all the control cows had a normal constricted cervix, whereas the cervices of the cows receiving relaxin were normal or near normal during the first estrous cycle but a small general build-up of relaxation was noticed in the second and third estrous cycles.

The mechanical dilator was used to determine the actual amount the cervix had relaxed. As shown by Table 2, the control cows and the experimental cows were dilated to approximately the same extent during the first and second estrous periods. The cervices of the experimental cows in the third estrous period were dilated an average of 1.27 inches and the controls averaged 0.85 inch. This gain probably is due to a build up of relaxin.

Five days post estrus the cervix of the control cows could not be dilated. The mechanical dilator could not be placed into the cervix. All cows receiving

TABLE 1

The effect of varying dosages of relaxin on cervical relaxation during three estrous cycles, measured by manual palpation

Cow No.	1st estrus 1-4-52 C	I	R[a]	Relaxin 250 G.P.U.	5 days post estrus C	I	R	10 days post estrus C	I	R	2nd estrus C	I	R	Relaxin 1,500 G.P.U.	5 days post estrus C	I	R	10 days post estrus C	I	R	3rd estrus C	I	R	Relaxin 8,500 G.P.U.	5 days post estrus C	I	R	10 days post estrus C	I	R
Experimental:																														
E 53			•	•			••		•		•			•			••••			•			••	•			•••••			•
E 55			•	•			••	•			•			•			••			•			•	•			•	•		
E 57			•	•			••		•				•	•			••••			•			••	•			••••			•
Control:																														
E 54			•		•			•					•			•		•				•			•			•		
E 59		•			•			•			•				•			•			•			•			•		
E 62		•			•			•			•				•			•			•				•			•		

[a] C = constricted, I = intermediate, R = relaxed.
Dots indicate degrees of relaxation.

TABLE 2

Amount of mechanical cervical dilation of cows treated with relaxin and untreated cows during 3 estrous cycles

Cow No.	1st estrus dilation	Relaxin treatment	5 days post estrus dilation	2nd estrus dilation	Relaxin treatment	5 days post estrus dilation	3rd estrus dilation	Relaxin treatment	5 days post estrus dilation
	(in.)	*(G.P.U.)*	*(in.)*	*(in.)*	*(G.P.U.)*	*(in.)*	*(in.)*	*(G.P.U.)*	*(in.)*
Experimental:									
E 53	0.87	250	1.06	0.62	1500	1.40	1.20	8500	1.50
E 55	0.81	250	0.87	0.75	1500	1.00	0.87	8500	0.90
E 57	0.87	250	0.87	0.90	1500	1.41	1.75	8500	1.61
Control:									
E 54	0.87	.....	none	0.62	.....	none	0.87	.....	none
E 59	0.75	.....	none	0.70	.....	none	0.87	.....	none
E 62	0.85	.....	none	0.62	.....	none	0.81	.....	none

relaxin were dilated, and with increasing dosages of relaxin an increased amount of cervical dilation was noted. In many cases by using relaxin the cervix could be dilated more than an inch, with an average of 0.90 in., with 250 G.P.U., 1.27 inches with 1,500 G.P.U., and 1.34 inches with 8,500 G.P.U.

Biopsies were taken from the cervix and uterus of each cow three times during each estrous cycle: at estrus, 5 days post estrus, and 10 days post estrus. Histological studies of the biopsies gave no indication of trauma from either relaxin or mechanical dilation.

DISCUSSION

The data suggest the possibility of mechanical and hormonal dilation of the cervix. The cervix was dilated with a mechanical dilator during estrus but could not be dilated post estrus unless the animal had been treated with relaxin. Relaxin definitely gave some relaxation of the muscles of the cervix; however, two cows, E 53 and E 57, responded more than did E 55.

Apparently the cervix can be dilated periodically without danger of traumatizing the epithelial wall. This is particularly important, for if the periodic collection of ova through the cervix is to become practicable, the cervix must be opened often without damaging the tissue.

SUMMARY

These experiments were conducted to determine the possibility of dilating the cervix to facilitate the ease of collecting ova.

The administration of relaxin in conjunction with an estrogen served to relax the cervical muscles. Estrogen alone as judged by the pilot cow gave no cervical relaxation. The minimum dosage of relaxin which gave cervical relaxation was 250 G.P.U.; the optimum, about 1,500 G.P.U. A suitable mechanical instrument was designed for dilating the cervix. However, the instrument must be used with the aid of relaxin when used 5 days post estrus. Histological studies of biopsies from the cervix and uterus indicated no signs of trauma from either the relaxin or the instrument.

ACKNOWLEDGMENTS

The authors express their appreciation to Robert L. Kroc, Director of Biological Research, Chilcott Laboratories, Morris Plains, N.J., for supplying the relaxin-containing extract, "Releasin," prepared from sow ovaries.

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PEOPLE and EVENTS
in the Dairy Science World

Shepherd Retires from Federal Service

JOSEPH B. SHEPHERD, Dairy Husbandman in the U. S. Department of Agriculture, retired May 15, 1953, after 36 years service. He has been a member of the Bureau of Dairy Industry since its inception in 1924. After graduating from the University of Nebraska he began his government career in 1917 in the Dairy Division of the Bureau of Animal Industry. Since 1945 he has had direct charge of the forage and pasture investigations in the Bureau at Beltsville. During recent years he has conducted investigations on pasture management and methods of forage preservation.

Mr. Shepherd was largely responsible for the development of the wilting method of making grass silage, which has received wide acceptance and has provided farmers with a low-cost, efficient method of harvesting hay crops for silage. He also developed a technique useful in studying the fundamental chemical and bacteriological changes which take place in silage making. The technique consists of using a $4' \times 8'$ glass-coated, steel silos which can be made airtight and fitted with portholes for sampling the forage.

Owens-Illinois to Build New Laboratory

Owens-Illinois Glass Co. has announced that it is starting construction of a new Technical Center at Toledo, Ohio, that will consolidate the company's general research program with the engineering activities of its various divisions.

In planning the building, versatility and adaptability have been emphasized. Lightweight interior partitions are demountable so that office space can be converted readily into laboratories, or vice versa, as the need arises. Rooms of various sizes can be created temporarily to meet the demands of the work.

The pilot plant area also is to be flexible in design and will have a minimum of partitions. One section, devoted to hot glass operations, will house several furnaces of 25-ton capacity each, forming machines and other installations similar to those found in plants serviced by Owens-Illinois research and engineering. The rest of the pilot plant area will house machine, electrical, and pattern shops and the areas devoted to developmental work.

There will be an employee recreation room and cafeteria in the basement and off the cafeteria a paved, landscaped courtyard. When completed, the Center will be occupied by 350 engineers, scientists and technicians.

Creameries of America Merges With Beatrice Foods

W. G. KARNES, president of Beatrice Foods Co., has announced the acquisition of Creameries of America, Inc. This company operates 42 plants located in California, Texas, New Mexico, Utah, Colorado, Idaho, and the Hawaiian Islands.

G. S. MCKENZIE, president and general manager of Creameries of America, will become a vice-president, district manager, and director of Beatrice Foods Co. The yearly sales of the merged companies will exceed $300,000,000. The distribution of sales will be approximately 37% milk, 18% ice cream, 18% specialty foods and services, 17% butter, 6% manufactured milk products, and 4% poultry and eggs. Beatrice Foods is the third largest dairy company in the United States.

News Items from Iowa State College

J. T. MILES joined the staff at Iowa State College as Assistant Professor of Dairy Husbandry July 1, 1953. Dr. Miles came to Iowa State from Mississippi State College. He received his Ph.D. degree from the Univ. of Wisconsin in 1951 and the B.S. and M.S. degrees from the Univ. of Tennessee. He has been herdsman at the U.S.D.A. station at Lewisburg, Tenn., and at the Univ. of Tennessee.

R. L. HANDWERK, who received his Ph.D. in Food Technology (Dairy Chemistry) from Iowa State College in 1950 and since has been with the U.S. Army Quartermaster Corps in Japan, has accepted a position in the Research Laboratory of Kraft Foods Co., at Glenview, Ill.

JOE ZALATEL, who received his M.S. in Dairy Industry at Iowa State College in 1950 and has been employed since then by the Dairy Husbandry Subsection of the Iowa Agricultural Experiment Station, has accepted a position in charge of chemical phases of the laboratory work at Mercy Hospital, Des Moines, Ia.

C. A. IVERSON, head of the Dairy Industry Department at Iowa State College, received an honorary Doctor of Science degree at South Dakota State College on June 8. Prof. Iverson received his B.S. degree from the same institution in 1915.

Happenings in Dairy Technology at Ohio State

Eight Dairy Technology scholarships have been awarded to graduates from high schools in northeastern Ohio who will enter The Ohio State Univ. in the fall to major in dairy tech-

nology. The scholarships carry stipends of $150 to $200 for the first year and have been made available by the Cleveland Dairy Technology Society, the Stark County Milk Distributors Assoc., and the milk dealers of the Akron area. The awards were based on written examinations and personal interviews.

The Department of Dairy Technology, working with the Ohio dairy industry, has sponsored two "Personnel and Efficiency Clinics." One was held in Toledo and one in Cincinnati. The meetings, designed for those in supervisory and management capacities, featured films, short talks by University and industry representatives, and group discussion. Subjects discussed were job selection and analysis, personnel selection and training, human relations, and personnel morale.

Creamery Package Announces New Branch Manager

F. S. BRUMLEY has been promoted from assistant manager to manager of the CP Atlanta Branch, replacing C. L. WEBB, who will become a special representative for national assignments. Mr. Brumley joined Creamery Package in 1943, after service with the Atlanta Health Department, where he supervised the inspection of dairy processing plants.

January and February Journal Issues Needed

The Association has gained members and subscribers at a faster rate than anticipated. As a result, there is an insufficient quantity of the January and February numbers of the Journal available to supply the needs for the remainder of this year. Any one having these issues who does not wish to retain them in his file will be doing the Association a service by sending them to P. R. ELLSWORTH, Secretary, American Dairy Science Association, The Ohio State University, Columbus 10, Ohio.

Plans for International Dairy Show Progressing

A tentative schedule of the judging program for the first International Dairy Show has been announced by W. E. OGILVIE, secretary-manager. The show will be held October 10 to 17 in the Chicago International Amphitheatre, which will accommodate 2,500 head of exhibition cattle. October 12, will be Junior Day, when 4-H and FFA-owned animals will be judged. Guernsey, Holstein-Friesian, Jersey, Ayrshire, Brown Swiss, and Milking Shorthorn breeds will be shown. A cash prize list of $5,000 will be offered for each breed, and many trophies will be awarded by the various breed associations.

The country's top cowboys will compete for points in a championship rodeo planned as the chief entertainment feature of the show.

News from Here and There

There were 1,628 persons officially registered for the 48th annual meeting of the A.D.S.A. at Madison, Wis., June 21-24. This is the largest group that has ever assembled for this meeting. The members of the staff at Wisconsin are to be commended for the excellent manner in which they arranged the details of the meeting, including the weather. Next year the annual meeting will be held at Penn State.

One of the older members attending the annual meeting was H. A. HARDING, a former head of the Department of Dairy Husbandry at the Univ. of Illinois. Dr. Harding, now 82, lives at R. R. 1, Boleys Harbor, Wis. His present hobby is carpentry.

The New York City Board of Health has ruled against the industry's request that the dating requirement of milk and cream be abolished. However, the Board has extended the time limit on milk sales to 54 hours. This 6-hour extension will enable store operators to sell on Monday milk that is dated Saturday.

B. E. PROCTOR of the Food Technology Department of M.I.T. and president of the Institute of Food Technology has been made a member of the board of National Phoenix Industries.

Increased emphasis is being placed on the selling of beer in food stores. In some stores more display space is alloted to this item than to milk. This is a trend that may have some effect upon future milk sales.

A new cheese made from ewe's milk has been introduced by Lekas and Drivas, Inc., 19 Roosevelt St., N. Y. City. The soft white cheese called Crem-O-Gal comes in 1- and 2-lb. tins.

To prove their point that people would buy more butter if the support price was removed, Frederick F. Lowenfels and Son, New York City, selected an "average" store for a test case. The chosen market has a usual Friday sale of about 500 lb. of butter and the same amount of margarine. The price of butter was lowered 24 cents below the market to 55 cents per pound and the Friday sale jumped to 4,975 lb. Gross sales at the store on this day increased from an average of $9,000 to $14,000. Margarine sales decreased to about 40 lb.

Pepperidge Farms, Inc., have opened a $500,000 bakery at Downers Grove, Ill., to serve customers throughout the Midwest with old-fashioned bread. Why don't our merchandising experts capitalize on the merits of old-fashioned bread and delicious ice cold milk, an ideal lunch on a hot summer day?

Superior croquet balls are now being made from wood flour, urea resin, phenolic resin,

and calcium stearate. These ingredients are blended in the dry state, then compressed and cured in a heated molding press. This is a development of the Chemical Division of the Borden Co.

The Wyandotte Chemical Corp. recently opened a new research laboratory at Wyandotte, Michigan. One wing is for management and the other for research. A feature of the laboratory building construction is its flexibility. The interior walls are so designed that they can be quickly moved except for the rooms requiring special equipment. All utility lines have paired outlets every 10 ft. These lines are located in a crawl space beneath the floor.

JUDSON MASON, director of dairy marketing for the Illinois Agricultural Association, recently returned from a 13-month stay in Europe as a dairy production specialist for the U. S. Dept. of Agriculture.

H. S. WILLARD of the Department of Animal Production of the Univ. of Wyoming is on leave to take a Point Four assignment in Jordan.

W. H. HASKELL, director of programs for Klenzade Products, Inc., has transferred the scene of his company operations from the Beloit offices to Ocala, Florida. For a number of years Dr. Haskell was connected with the U. S. Public Health Service.

GEORGE HYATT, JR., former farm manager and editorial advisor for Hoard's Dairyman and currently dairy specialist in the Extension Service at North Carolina State College, has been named to succeed JOHN A. AREY, who has been in charge of the dairy extension program.

New Column to be Added

In keeping with the desire of a majority of the members to broaden the scope of our present Journal, a new department entitled "Letters to the Editor" will be started in an early issue.

The purpose of the column will be to publish letters of constructive criticism regarding specific articles that have been published in the Journal, the methods of handling our annual meeting, the type of programs arranged by the committee, and suggestions regarding the Journal. In addition, this column will serve as an outlet for short articles dealing with some experiment, method, or equipment which the author would like to have published but which does not justify publication as an article in the Journal. To be published, a letter must be signed. It is hoped that members will contribute generously to this new department.

Editorial Board to be Established

At the Madison meeting of the A.D.S.A., the Official Board voted to establish a plan whereby an editorial board will be selected for the purpose of passing judgment on all manuscripts sent to the editor for publication. The members of this board will be chosen in such a manner as to represent each of the specialized fields covered by the Journal. Men recognized as authorities in their fields and who are well qualified to evaluate the merits of an article will be given the distinction of serving on this board. The existence of this board will not preclude the possibility of other members of the Association being invited, either by a member of the board or by the editor, to review manuscripts.

W. O. Nelson Appointed Abstract Editor

In order to render better service to the readers of the Journal, plans are being made to increase the number of journals abstracted and to shorten the time between publication of an article and the printing of the abstract in the Journal. Instead of sending abstracts to section editors, as has been the practice in the past, one person will serve as editor for all abstracts. DR. W. O. NELSON of the Dept. of Dairy Science at the Univ. of Illinois has consented to accept this responsibility.

Instructions to Authors to be Published

In order to give authors a clearer understanding of the editorial policy of the Journal, a detailed article will be published in an early issue setting forth the rules that should be followed in preparing manuscripts for publication. A limited number of reprints will be available for distribution to departments and government agencies.

National Dairy Scientists Honored

In a ceremony held at the National Dairy Research Laboratories, Inc., in Oakdale, Long Island, five members of the Laboratory staff were honored on June 12, 1953, by their associates for outstanding contributions to scientific progress. The function, a Recognition Assembly, was presided over by DR. L. B. HITCHCOCK, president of the Laboratories, and DR. A. H. JOHNSON, vice-president of the Laboratories, presented the awards.

Two research fellowships were established giving greater freedom of action to the scientists so honored. The fields of investigation of the recipients are open to their own choosing. The two Fellows appointed were E. H. FREUND and G. H. HAUGAARD. Dr. Freund has had extensive experience in the fields of organic chemistry with particular emphasis on synthetic fibers, cellulose acetate films, plastics, emulsifiers, antioxidants and lactose derivatives. Dr. Haugaard is well known for his role in the identification of protein-bound sugars as well as his work on amino acids, carbohydrates, the glass electrode, and chromatography. He was one of the first to perform chromatographic separation of ions under the influence of an applied potential.

For outstanding research in the application of enzymology to human and animal nutrition, the Achievement Award was shared by E. G. STIMPSON and HAROLD YOUNG. Dr. Stimpson, director of the Division of Biochemistry, has been associated with the Laboratories for 16 years, during which time he has been concerned with research on enzymes, ion-exchange, and animal nutrition. Mr. Young, who has been associated with the Laboratories for 5 years, is particularly active in studying fermentations.

For his outstanding accomplishments in the field of detergents and sanitizers, the Senior Scientists of the Laboratories have elected H. G. HARDING to that distinguished rank. Dr. Harding is a pre-eminent authority in this field.

Completed Theses

M.S. Degree:

ROBERT C. FINCHAM—Response of dairy heifers to aureomycin feeding. Iowa State College.

ROBERT B. HEGLAND—Factors affecting reflex closure of the esophageal groove in the calf. Iowa State College.

FRANK B. YOUNG—Hydrogenated soybean oil and dried whey product in whole milk replacements for dairy calves. Iowa State College.

S. J. PEARCE—Factors affecting the breaking of milk and cream emulsions by surface active agents. Oregon State College.

W. E. DENT—Nutritional studies of copper and cobalt in cattle in the Oregon coastal region. Oregon State College.

RICHARD H. ANDREWS—The destruction of psychrophilic bacteria in milk. University of Illinois.

Ph.D. Degree:

GLENN A. CLABAUGH—Non-sporulating obligately anaerobic bacteria in dairy products. Iowa State College.

MAURICE R. LAMBERT—Lipid requirements of the young dairy calf. Iowa State College.

VERNER H. NIELSEN—Properties and distribution of casein and non-casein proteins, calcium and phosphorus in skimmilk, buttermilk and butterserum from the same whole milk. Iowa State College.

W. CARL VAN DER ZANT—Proteolytic enzyme system of *Streptococcus lactis*. Iowa State College.

Recent Deaths

CARROLL C. FLORA, Associate Professor of Dairy Husbandry at Virginia Polytechnic Institute, died May 20 at the age of 43. Dr. Flora, a graduate of V. P. I., took his graduate work at Penn. State. He served in the Sanitary Corps during World War II.

HARRY W. CAVE, aged 63, former head of the Department of Dairy Husbandry at Oklahoma A. and M. College and a past president of A.D.S.A., died June 23. He had been in poor health for some time. Professor Cave obtained his B.S. degree from Iowa State College and his M.S. degree from Kansas State College. He was connected with the U. S. Dept. of Agriculture from 1916 to 1918, after which he returned to Kansas State. In 1939 he became head of the department at Oklahoma.

Progress and Advancement

A Guest Editorial

The struggle for improvement in the dairy industry has brought about a complexity of distortions which have assumed such proportions as to change the entire outward form of the industry. Some of these have been created forces from within and some have been caused by pressures from without. Whether we call these changes normal or unusual depends entirely upon our point of view.

The same scientists who brought out the refinements and improvements in the dairy industry have also developed substitutes which threaten the composition of the ultimate "dairy" product itself. The same engineers whose talents have produced highly efficient sanitary equipment have turned their eyes to other products which could be processed in the same units.

George D. Armerding

As progress and advancement move along the highways of time, the forces developed are relentless. Nothing must stand in the way. The signals are green; the traffic is moving. Even *sacred cows* cannot survive against the flow of this stream. We need not despair. This is not an uncontrolled down hill run-a-way. It is the forward movement of progress and we must advance with it or be ground under and destroyed.

The distortions that we frowned upon as monstrosities are but streamlining changes. The pressures that threatened to explode are simply the stepped up driving forces to speed us on our way. Shall we jettison the science that seems to threaten the old ship? Shall we legislate our competitors down to our speed? Such actions would move us off the highways of progress and leave us stranded in the ruts of yesterday's by-ways. We must progress. We must advance.

GEORGE D. ARMERDING
Mojonnier Bros. Co.

Labor Developments

July 5 was the last day for milk plant operations and Sunday delivery in Washington, D. C. Wage rates for employees and wholesale routemen were increased 3 dollars per week. Retail routemen's base wages and guarantees were increased 5 dollars per week. Sunday delivery has been discontinued in about half the larger markets of the country.

After several weeks of negotiations, the weekly wage rate of employees of Chicago dairies was increased 7 dollars, effective May 1, this year. An additional 3 dollars per week becomes effective May 1, 1954. The dealers rejected a demand of union officials that the plants employ a proportionate number of men to volume of milk processed, as existed the last week in January, when the present no-Sunday processing and delivery plan went into effect.

Pay increases and shorter work weeks have been granted labor in several other cities throughout the country. The ever increasing cost of giving milk its form and place utility emphasize even more the need for research workers to develop a satisfactory process for sterilizing fresh milk and methods for aseptically canning or bottling. When this is accomplished—and there is every reason to believe that it will be done in the not too distant future --the present system of pasteurizing, bottling, and distributing market milk will become obsolete.

VOLUME XXXVI JULY, 1953 NUMBER 7

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BUTTER

H. A. HOLLENDER, SECTION EDITOR

440. The vitamin A potency of New Zealand butter. A. K. R. McDOWELL and F. H. McDOWALL, Dairy Research Inst., Palmerston North, New Zealand. J. Dairy Research, **20**, 1: 76-100. Feb., 1953.

Results of the first extensive study of the vitamin A potency of New Zealand butter are presented. The report is based on the analysis of 1,517 samples of fresh butter and 670 samples of stored butter. Samples were taken from 20 representative factories at bi-weekly intervals for 3 successive yr. (1946-48).

The mean vitamin A potency of New Zealand butter for the 3-yr. period was 36.9 I.U/g., or 16,740 I.U./lb., allowing for a 7.0% loss of vitamin A in analysis. Seasonal and regional variations in carotene and vitamin A contents are presented. Seasonal changes in carotene and vitamin A contents paralleled seasonal changes in iodine values.

No significant losses occurred in vitamin A potency of butter samples stored for 8 mo. at 14° F., even though some contained appreciable amounts of iron and copper. E. L. Thomas

441. Process for the preparation of a stable butter. F. D. TOLLENAAR (assignor to de Nederlandse Organisatie voor Toegepast-Natuurwetenschappelijk Onderzoek ten behoeve van de Voeding). U. S. Patent 2,638,420. 15 claims. May 12, 1953. Official Gaz. U. S. Pat. Office, **670**, 2: 531. 1953.

Rancidity in butter is prevented by the incorporation of dialkyldithiocarbamic acid and its salts. R. Whitaker

442. The nature and the mechanism of the process (of butter formation). M. KAZANSKII. Molochnaya Prom., **14**, 1: 19-21. 1953. (Chem. Abstr., **47**: 4006c.) S. Patton

CHEESE

S. L. TUCKEY, SECTION EDITOR

443. Studies on a washed curd variety of cheese. I. The influence of different types of starter. JOAN WHITMORE, Dairy Dept., The Univ. of Reading. J. Dairy Research, **20**, 1: 108-114. Feb., 1953.

The manufacturing procedure for a washed curd variety of cheese is described. The body of the ripened cheese resembles that of the Swiss varieties.

Grading data on cheese from split vats of milk are presented. One vat in each trial was inoculated with a lactic starter and the other with a Swiss starter; otherwise the treatments were identical.

A significantly higher number of good-flavored cheese resulted when the lactic starter was used. Bitterness and meatiness were more evident in cheese made with Swiss starter. Although better eye development was obtained when Swiss starter was used, there was little correlation between eye formation and flavor scores. E. L. Thomas

444. The role of fungi in cheese ripening. F. J. BABEL. Econ. Botany, **7**: 27-42. 1953 (Chem. Abstr., **47**: 4006d.) S. Patton

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

445. The associative growth of Bacillus cereus and Streptococcus lactis in milk. ELLEN I. GARVIE and M. JEAN STONE, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **20**, 1: 29-35. Feb., 1953.

Regardless of the initial proportions of *Bacillus cereus* and *Streptococcus lactis* when the organisms were grown in pasteurized milk at 22° C., the streptococci greatly outnumbered the spore-former at the endpoint of the clot-on-boiling test. Broken cream failed to occur in tubes with a high proportion of acid-producing organisms. It is suggested that the prevalence of broken cream in pasteurized milk may be the result of heat destruction of lactic streptococci. Mixed milk cultures of these organisms in the ratio of 100:1 showed increasing incidence of broken cream and proportion of the spore-former with increasing incubation temperature between 15 and 22° C. It is concluded that the absence of broken cream at low temperatures is solely a function of temperature, and that at temperatures favorable

to the development of *B. cereus* the presence of lactic streptococci was neither inhibitory nor stimulatory. J. C. Olson, Jr.

446. The inhibition of microorganisms by raw milk. I. The occurrence of inhibitory and stimulatory phenomena. Methods of estimation. J. E. Auclair and A. Hirsch, Station centrale de Microbiologie et Recherches laitieres, I.N.R.A., Paris; and Natl. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **20**, 1: 45-59. Feb., 1953.

Using the diffusion cup method and *Micrococcus lysodeikticus* as test organism, the antibacterial property of milk was clearly differentiated from the action of lysozyme. Plots of acid production by *Streptococcus pyogenes* versus dilutions of heat-treated milk (58° C. for 20 min.) gave curves indicative of the interaction of a stimulatory and two inhibitory substances. The difference in pH and heat stabilities between the inhibitors L_1 and L_2 serves as the basis of a method for their separate estimation in unknown samples. L_1 and the stimulatory property occur mainly in colostrum, whereas L_2 occurs mainly in milk. Anaerobiosis and cysteine abolish the inhibitory properties but do not affect the stimulator.

J. C. Olson, Jr.

447. Some group N streptococci isolated from raw milk. Ellen I. Garvie, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **20**, 1: 41-44. Feb., 1953.

Physiological and serological tests of cultures isolated from raw milk showed the presence of group N streptococci other than *Streptococcus lactis* or *S. cremoris*. These strains appeared with greatest frequency in milk incubated at 22° C. and contributed to its spoilage. In their response to temperature, pH, and salt, the cultures resemble *S. cremoris*, but they ferment an even wider range of carbohydrates than does *S. lactis*. J. C. Olson, Jr.

448. The effect of storage at different relative humidities on the survival of microorganisms in milk powder and in pure cultures dried in milk. Constance Higginbottom, The Hannah Dairy Research Inst., Kirkhill, Ayr. J. Dairy Research, **20**, 1: 65-75. Feb., 1953.

In general, the survival of the microflora during storage of 2 spray-dried milks was an inverse function of the relative humidity. However, maximum survival occurred at about 10% R.H. and decreased at lower moisture contents. After 48 wk. storage at 37° C., streptococci and micrococci predominated at relative humidities below 50%; at 30° C. the streptococci were supplanted by non-spore-forming rods. At higher moisture contents the flora consisted almost exclusively of spore-forming bacilli. It was observed in the dried milks and confirmed in pure culture studies with *Bacillus subtilis* that bacterial spores are more sensitive to extremely low humidity during storage than are those nonsporing bacteria present in the powder.

J. C. Olson, Jr.

449. The so-called "nascent" bacteriophage phenomenon. H. R. Whitehead, Anne East, and Lola McIntosh, The Dairy Research Inst., Palmerston North, New Zealand. J. Dairy Research, **20**, 1: 60-64. Feb., 1953.

The apparent lysis of a strain of *Streptococcus lactis* by a "nascent" heterologous bacteriophage was shown not to be limited to the time of liberation of the phage from the host organism. The effect can be brought about by the phage in the absence of the homologous bacteria and is actually an inhibition of the bacteria caused by a high concentration of the phage. The extent of inhibition of a strain of *S. lactis* indicates the closeness of its relationship to the homologous strain.

J. C. Olson, Jr.

450. The heat resistance of Streptococcus faecalis. H. R. White, King's College of Household and Social Science, London, W. 8. J. Gen. Microbiol., **8**, 1: 27-37. Feb., 1953.

Results of thermal death-time studies using *S. faecalis* suspended in saline indicated that the plot of log survivors/time was not a straight line. The age of culture markedly influenced the heat resistance of the 3 strains studied in detail. The heat resistance of these cultures was shown to be changed at various phases of the growth curve, and the breaks in the thermal death curves appeared to be greatest in extremely young cultures in which populations probably were not physiologically homogeneous. High incubation temperatures resulted in increased heat resistance in all but the youngest cultures. J. J. Jezeski

451. The effect of carbon dioxide pressure on a bacterial decarboxylase system. W. H. Oliver, Dairy Research Inst., Palmerston North, New Zealand. J. Gen. Microbiol., **8**, 1: 38-44. Feb., 1953.

The decarboxylase activity of *Streptococcus diacetilactis* depended upon the amount of CO_2 in the culture atmosphere, an increase of CO_2 in the atmosphere repressing the formation of CO_2 by the culture. Other products of the reaction appeared to decrease the amount of CO_2 formed. These results are explained on the basis of the law of mass action. Some data are presented which indicate that increased CO_2 pressures influence the amount of bacterial decarboxylase formed; however, no data are presented which overrule the possible effect of other factors in the environment on the activity of the decarboxylase system studied.

J. J. Jezeski

452. Lactate-oxidizing systems in the mycobacteria. N. L. Edson and F. B. Cousins. Nature, **171**, 4355: 702. 1953.

Of several mycobacteria studied, the most active L-lactate oxidizing enzyme preparation was obtained from *M. smegmatis*. An orange-yellow pigment, present in the partially purified enzyme, is progressively removed as the enzyme is purified. The authors found that H_2O_2 is produced when the lactate radical is oxidized and believe an autoxidizable flavo-protein containing FAD (flavin-adenine-dinucleotide) takes part in the reaction. There was no evidence of a DPN (dephospho-pyridine nucleotide)-linked anaerobic dehydrogenase being produced by the mycobacteria studied. R. Whitaker

453. An improved medium for lactobacilli. Mary Briggs, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **20**, 1: 36-40. Feb., 1953.

A tomato glucose broth containing Tween 80 and starch supports excellent growth of all lactobacilli tested, including the more exacting species. It is recommended for the study of physiological and biochemical characteristics of the lactobacilli and is being used by the authors in a reclassification of the group.

J. C. Olson, Jr.

454. Action of hydrogen peroxide on Brucella in milk. Giovanni Giolitti. Igiene e sanita pubblica, **8**: 433-443. 1952. (Chem. Abstr., **47**: 3928b.) S. Patton

DAIRY CHEMISTRY

STUART PATTON, SECTION EDITOR

455. The properties of New Zealand butters and butterfats. III. Seasonal variations in the nature of the unsaturated acids of butterfat as estimated by spectrophotometric methods. A. K. R. McDowell, Dairy Research Inst., Palmerston North, N. Z. J. Dairy Research, **20**, 1: 101-107. Feb., 1953.

Results are presented of a study of the relationship between iodine value and type of unsaturated acid in butterfat as estimated by the absorption spectrophotometric method. Seasonal variations in iodine values were closely related to changes in oleic acid content. Conjugated dienoic acids, though present in relatively small amounts, showed a somewhat similar seasonal trend. Values for nonconjugated dienoic acids were low and fluctuated considerably from month to month. Only traces of conjugated trienoic acids were present. The content of nonconjugated trienoic acids ranged from 0.5 to 1.0%. No trace of conjugated tetraenoic acid was found in any of the samples examined, but 0.3 to 0.4% of nonconjugated tetraenoic acid was present in all samples.

E. L. Thomas

456. Chemical interactions of amino compounds and sugars. VI. The repeating unit in browning polymers. M. L. Wolfrom, R. C. Sebllicht, O. W. Langer, Jr., and C. S. Rooney, Dept. of Chemistry, The Ohio State Univ., Columbus. J. Am. Chem. Soc., **75**, 4: 1013. Apr., 1953.

Procedure is given for the preparation and purification of a brown solid, the result of reactions between glycine and L-arabinose, D-xylose and D-glucose. The reactants were alternatively labeled with radioactive carbon which made it possible to derive empirical formulas for the polymers from consideration of the activities of the polymers and reacting substances. The empirical formulas so calculated were $C_{7.0}H_{6.4}O_{2.0}N_{0.5}(CH_3)(CO_2H)_{0.5}$ for the glycine reaction product from D-glucose and $C_{5.0}H_{4.3}O_{1.5}N_{0.5}(CH_3)(CO_2H)_{0.5}$ for each of the products from the pentoses.

The polymers appeared to arise from an equimolar reaction between the reducing sugar and the amino acid without carbon chain scission of the sugar, with partial decarboxylation of the amino acid and with nearly complete trimolar dehydration of the sugar. J. Tobias

457. Amino acid composition of γ-casein. W. G. Gordon, W. F. Semmett, and Maurice Bender, Eastern Regional Lab., Philadelphia, Pa. J. Am. Chem. Soc., **75**, 7: 1678-1679. 1953.

The distribution of amino acids in γ-casein resembled in general that in β-casein. The difference in their properties may be accounted for in the differences in their side chain groups, particularly the phosphoserine group. Protein solubility, electrophoretic mobility, and specific volume of γ-casein were related to its amino acid composition. J. Tobias

458. The terminal amino groups of α- and β-caseins. Edward F. Mellon, Alfred H. Korn, and Sam R. Hoover. J. Am. Chem. Soc., **75**, 7: 1675-1678. 1953.

Dinitrofluorobenzene was used to label the amino groups of the α- and β-fractions of casein and the hydrolyzed proteins were assayed for the various dinitrophenyl amino acids by chromatography. Only lysine and arginine were found to occupy terminal positions in the purified caseins. α-casein contained 7 terminal arginyl residues for each terminal lysyl residue, whereas the ratio in β-casein was 2 to 1. Details of procedure are given. J. Tobias

459. Milk product containing milk fat, and process of producing same. C. A. Kempf, J. S. Neidlinger, A. P. Stewart, Jr., and P. F. Sharp (assignors to Golden State Co., Ltd.). U. S. Patent 2,638,418. 26 claims. May 12, 1953. Official Gaz. U. S. Pat. Office, **670**, 2: 530. 1953.

The natural lipase of milk is allowed to hydrolyze from 15 to 40% of free fatty acids

of milk fat at 30 to 50° F. Approximately 30 to 45 % of the volatile water-soluble free fatty acids and 15 to 25% of volatile water-insoluble free fatty acids then are removed. The resulting product finds use as a food ingredient having a strong milk flavor. R. Whitaker

460. Human milk. Yield, proximate principles and inorganic constituents. S. D. Morrison, Farnham Royal, Bucks. Commonwealth Agr. Bur. 91 pp. (Chem. Abstr., **47**: 3960h.) Apr., 1953. S. Patton

461. Refractometric method of determination of sugar content of ice cream. M. Snigur. Molochnaya Prom. **14**, 1: 23-25. 1953. (Chem. Abstr., **47**: 4006b.) Apr., 1953. S. Patton

DAIRY ENGINEERING

C. W. HALL, SECTION EDITOR

462. Ice cream dispensing machine. A. J. Murphy (assignor of ½ to S. Lawrence Atkins). U. S. Patent 2,638,064. 8 claims. May 12, 1953. Official Gaz. U. S. Pat. Office, **670**, 2: 428. 1953.

Bulk ice cream in a vertical cylindrical shaped housing is pressed downward against the bottom where a pusher bar, advancing from one side, cuts off an individual serving and moves it underneath a hemispherical mold. The mold moves downward, shaping the ice cream and ejecting it ready for serving. The entire mechanism is operated by one lever.

R. Whitaker

463. Mechanism for making and depositing balls of ice cream or the like. R. G. Tarr (assignor of ½ to Jack C. Webb, and ½ to Taft Moody). U. S. Patent 2,638,065. 10 claims. May 12, 1953. Official Gaz. U. S. Pat. Office, **670**, 2: 428. 1953.

A device for automatically molding and dispensing individual ball-shaped portions of ice cream from bulk ice cream is described. A knife, shaped to fit the mold, releases the ice cream as the cup or mold is rotated.

R. Whitaker

464. Machine for coating confections. E. J. Otken (assignor to Good Humor Corp.). U. S. Patent 2,638,071. 38 claims. May 12, 1953. Official Gaz. U. S. Pat. Office, **670**, 2: 429. 1953.

Frozen confections are coated with nuts or other materials by inserting a rack holding the confections in this drum-shaped device. As the device rotates, the coating material is carried upward and spilled down over the confections, the excess falling to the bottom of the drum for further recirculation. R. Whitaker

465. Method and apparatus for releasing frozen confections from molds. K. Eftihios. U. S. Patent 2,637,668. 8 claims. May 5, 1953. Official Gaz. U. S. Pat. Office, **670**, 1: 243. 1953.

Confections frozen in molds in a brine tank are released from the molds by this apparatus, which consists of a tank of circulating warm water with a standpipe to insure a constant level. Mounted on the bottom of the tank are several legs, each surrounded by extended coiled springs supporting a platform. The mold to be defrosted is placed on the platform which is normally held at a high level by the springs, and then pushed downward until the platform rests on the legs. When the downward pressure is discontinued, the platform rises and the mold is removed from the bath. R. Whitaker

466. Receptacle supported stationary rest and wiper for milk cans. P. P. Prevost. U. S. Patent 2,636,205. 3 claims. April 28, 1953. Official Gaz. U. S. Pat. Office, **669**, 4: 1021. 1953.

A semicircular shaped bracket is attached to the inside surface of a tank in which cans of milk are cooled. The bracket facilitates removal of the can from the tank by partially supporting the can and at the same time wiping one side of the can as it slides out of the bracket. R. Whitaker

467. Apparatus for heating fluids, particularly foodstuffs. A. H. Brown, P. W. Kilpatrick, and M. E. Lazar (assignors to the U.S.A. as represented by the Sec. of Agriculture). U. S. Patent 2,636,430. 1 claim. April 28, 1953. Official Gaz. U. S. Pat. Office, **669**, 4: 1083. 1953.

A high-temperature short-time heater for fluid food products which tend to foul tubular and plate-type heaters. It consists of a cylindrical chamber through which the food passes. Steam is introduced into the product through an injector placed near the inlet end of the chamber and so positioned that the liquid and steam spiral through the chamber in turbulent flow. The flow rate and chamber size are adjusted to provide a heating period of 1 sec. or less.

R. Whitaker

468. Sanitary pump with infinitely variable output. G. R. Funk (assignor to Waukesha Foundry Co.). U. S. Patent 2,636,440. 4 claims. April 28, 1953. Official Gaz. U. S. Pat. Office, **669**, 4: 1087. 1953.

A sanitary pump of the vane type has a built-in by-pass which returns a portion of the product to the pump intake. A diaphragm valve, operated by a hand screw, regulates the flow through the by-pass and this in turn determines the pump output. R. Whitaker

469. Can cooker. P. Carvallo. U. S. Patent 2,632,378. 11 claims. March 24, 1953. Official Gaz. U. S. Pat. Office, **668**, 4: 983. 1953.

A continuous sterilizer consists of an endless

conveyor, equipped with metal baskets, which travels through 3 temperature zones: heating, holding, and cooling. The cans of product are loaded into the baskets and are sterilized by passage through the 3 zones. The baskets can be introduced and removed through star wheel locks to permit sterilization under pressure. The apparatus also may be made tall enough to keep the pressure of the holding zone above the flash point by passing the conveyor in and out through a tall regenerative type of heater and cooler. Several variations of the basic design are given. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

470. Operating ratios. L. C. THOMSEN, Dept. of Dairy Industry, Univ. of Wis., Milk Plant Monthly, **42**, 4: 23-25. April, 1953.

One approach to any analysis of business operations, as revealed by financial statements, is the use of ratios. It consists of comparing 2 sets of figures from a financial statement in order to ascertain their relationship. Operating ratios have several purposes: They may be used to eliminate the need for lengthy examinations of cumbersome figures in financial statements by stockholders or executives who are not trained accountants; they help to establish bank credit; they simplify periodic comparisons of operating results; comparisons among companies in the same business may be made more readily; they serve as an aid in formulating operating policies for the given company; and, if appropriate ratios are available for a sufficient number of companies and over a sufficiently long period, they may be helpful for formulating industry wide policies. Tables are presented showing assets to liability ratios, profits to sales and worth, net worth to operations, average on labor costs, and fixed assets to other factors. These are based on a 5-yr. study of 12 large corporations. C. J. Babcock

471. Orange ups profits. Anonymous. Milk Plant Monthly, **42**, 4: 40-41, 52. Apr., 1953.

There is more profit in 1 qt. of dairy orange than in 10 qt. of milk. The following are suggested means of selling dairy orange: Pass out qt. samples to as many customers as possible; tie in a qt. of orange for a penny with purchase of a qt. at the regular price; get the routeman's cooperation with a premium contest; for a specified number of bottle caps and a small cash payment, give the customer a useful gadget; devote part of your regular program time or display space to orange promotion; and stress point-of-sale advertising.

C. J. Babcock

472. Functional modernism in New Zealand. THOMAS P. J. TWOMEY, New Zealand Dept. of Agriculture. Milk Dealer, **42**, 7: 50-51, 70. Apr., 1953.

The $1,500,000 milk pasteurizing plant in Auckland, N. Z., is operated by the Auckland Milk Treatment Corp., which is a government financed public utility corporation. The plant occupies 40,000 sq. ft.; it segregrates processing functions and utilizes natural light to the maximum extent. It provides 20,000 to 25,000 gal. of bottled pasteurized milk daily, most of which is distributed through private vendors. Whether home delivered or sold at a shop dairy, the milk retails at just under 7.5¢ per qt. This is a subsidized price, which applies throughout most of New Zealand. Free of subsidy, the price would be approximately 10.5¢ per qt. The milk averages 4.4% butterfat. The country's legal minimum is 3.25% but the Auckland Metropolitan Milk Board stipulates a minimum content of 3.5%. The bacterial quality of the milk is satisfactory. C. J. Babcock

473. New methods in handling milk cases. ROBERT W. MOJONNIER, Mojonnier Brothers, Co., Chicago. Milk Dealer, **42**, 7: 54-55, 87-89. Apr., 1953.

The following methods of handling milk cases are discussed: pre-stacking of loads for retail routes for loading by the drivers; automatic stacking of cases; use of floor chains and automatic stackers in cold rooms for handling of cases in stacks; use of floor chain and stackers in docks for stack handling of cases for wholesale trucks; and automatic unstacking of cases. A sketch is given showing a hypothetical plant using *(a)* conventional "belt system" of loading; *(b)* original driver loading system for use in mild climates with cases iced and stacked outdoors, bottling done during the night; and *(c)* same system converted to any climate by enclosing the stacking area and turning it into a cold room, bottling done day or night. It requires 27.5 man-hr. labor to load out cases under method *a* as compared to 19.5 hr. under method *b* or *c*. An automatic stacker can handle 15 cases per min. indefinitely, whereas a good man can stack only 10 cases per min. for only a short time. The case-handling labor for load-out using floor chains and stackers in cold rooms is 24.5 man-hr. as compared to 10 man-hr. for wholesale docks with stacker and floor chains.

C. J. Babcock

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

474. Isolation and characterization of constituents of alfalfa wax. E. H. BLAIR, H. L. MITCHELL, and R. E. SILKER. Kans. Agr. Expt. Sta., Manhattan. Ind. Eng. Chem., **45**, 5: 1104-1106.

Alfalfa wax can be prepared from a carotene concentrate of alfalfa meal. This report is concerned with the separation and identification

of the components of this wax. It contained esters, paraffins, and alcohols. This study indicated that 1 T. of dehydrated alfalfa meal would yield 2.2 lb. of wax consisting of about 30% esters and 70% paraffins, as well as 13 lb. of chlorophyll concentrate containing 0.2 lb. of a wax alcohol. B. H. Webb

ICE CREAM

C. D. DAHLE, SECTION EDITOR

475. Miscellaneous factors which may affect quality in ice cream. P. S. LUCAS and ROGER WILKOWSKI, Mich. State Coll., East Lansing. Mich Agr. Expt. Sta. Quart. Bull. **35**: 317-323. 1953.

Five factors which may affect the quality of ice cream are pasteurization temperatures, homogenization pressures, homogenization temperatures, the aging period, and temperature in the hardening room. Quality was determined by acidity, pH, viscosity, surface tension, protein stability as determined by the alcohol member, fat globule size, clumping, whipping quality, body and texture score, melt-down characteristics, and ice crystal size. The mixes contained 12% butterfat, 11% serum solids, sweetener to the equivalent of 15%, and 0.3% stabilizer.

After pasteurization at 150, 160, and 170° F. for 30 min. the mixes were subjected to the above tests, and in all except one, temperature of pasteurization showed no noticeable effect on any of the properties of the ice cream. The exception was viscosity, which in 2 batches was increased after homogenization and in 3 others was decreased.

Ice cream was homogenized at pressures which were increased in 500 lb. increments, from 1,500 to 3,500 p.s.i. These batches had been pasteurized at 150° F. for 30 min., cooled, and aged for 24 hr. at 40° F. before freezing. Viscosity was increased appreciably by an increase in pressure. Viscosity determinations were made with a MacMichael viscosimeter.

In studying homogenization temperature, a single stage homogenizer operating at 2,500 p.s.i. was used. The temperature of the mixes at the time of homogenization was 110, 130, 150, and 170° F. Changes in temperature affected viscosity and clumping. For each increase in temperature, there was a decrease in viscosity. The same was true for clumping.

Mix was aged 0, 4, 24, and 48 hr. An increase in the aging period increased viscosity, slightly bettered the quality of the ice cream, and increased the speed of melt-down. Other properties were unaffected.

The effect of hardening room temperature was noted with both continuous and batch-frozen ice cream on score, melt-down, and ice crystal size. Temperatures of 0, —10, and —20° F. were used for hardening. The lower temperatures proved more advantageous for batch-frozen than for continuous frozen ice cream. The continuous-frozen ice cream was made up of smaller crystals than the batch-frozen product. Consequently, it was less coarse and melted less rapidly owing to the insulating effect provided by the smaller air cells.

Roger W. Hunt

MILK AND CREAM

E. O. HERREID, SECTION EDITOR

476. How to prevent off-flavors in milk. J. A. NELSON, Montana State College, Bozeman. Milk Plant Monthly, **42**, 4: 42, 46-48. Apr., 1953.

Milk flavor defects are discussed under 4 general groupings: (1) those caused by faulty production, i.e., cowy, barny, unclean, feed, high acid, malty, musty, and weedy; (2) milk processing defects, i.e., heated or cooked, metallic, oily, oxidized, stale, and tallowy; (3) absorbed flavors, i.e., foreign, disinfectant; and (4) miscellaneous flavor defects, such as bitter, rancid, flat, and salty. Since the flavor and quality of milk may be jeopardized by faulty production or processing, constant checks should be made in order to maintain a high standard product. C. J. Babcock

477. Short-time freezer storage of milk for home use. G. M. TROUT, J. C. BOYD, and C. K. SMITH, Mich. State College, East Lansing. Mich. Agr. Expt. Sta. Quart. Bull. **35**: 359-372. 1953.

Milk used in these studies was pasteurized, homogenized, and packaged in qt. paper containers. The packages were removed directly from filling machines of 3 local dairies. Part of another lot in glass bottles was transferred to paper cartons in the laboratory for freezing, and some was stored in the original glass bottles.

All milk held for 6 wk. at —20° F. was considered good (37-39 score). Three trials were made in which milk was held at 33 ± 1° F. for 14, 28, and 42 d., respectively. The keeping quality ranged from excellent to poor in the same order as the length of storage. In another study identical filler-run qt. samples of milk were stored at 40 ± 1° F. The beverage life of the samples rarely exceeded 14 d. However, milk from different plants varied markedly.

Roger W. Hunt

478. Container. J. C. GRAVENO (assignor of ½ to Joseph E. Stern). U. S. Patent 2,634,896. 2 claims. Apr. 14, 1953. Official Gaz. U. S. Pat. Office, **669**, 2: 500. 1953.

A single service paper container of the slanting-top type for milk and other liquid products is described. The container is characterized by having the top so folded that either end of the slanting top may be pulled out to form a pouring spout. The container is sealed by a staple and the pouring spout is formed

by tearing slightly the fold of paper which forms the ridge of the container.

R. Whitaker

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

479. Concerning the interpretation of observations obtained from records and experiments on milking dairy cows. C. P. Cox, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **20**, 1: 1-12. Feb., 1953.

A method of analysis to be utilized in developing physiological concepts on the relationships of the rates of secretion of the main constituents of milk is presented. Because of changing rates of secretions of butterfat, solids-not-fat, and the aqueous phase, Cox feels it much more informative to express rates of secretion absolutely rather than on a percentile basis, as is commonly done for butterfat. Changes in the per cent values are brought about by changes in either the numerator or denominator or both, and, conversely, there may be changes in both without reflexion in the per cent value.

Three levels of roughage were fed to groups of cows. It was found, by this analytical approach, that lowering the roughage per head per day from 18 to 6 lb. brought about an actual increase in the aqueous phase with a slight fat to solids-not-fat shift which resulted in a slight butterfat per cent depression. With a drop to 2 lb./day roughage, total yield decreased, the aqueous phase and solids-not-fat to a lesser degree than fat, resulting in a further fat per cent depression.

The inverse relationship of butterfat per cent and increase in milk yield, as recorded by Edwards, was examined. The results of applying this method of analysis to this study show that the lessened fat per cent is due almost entirely to an increased milk serum fraction with actually a small increase in fat also.

The formula is applied to an analysis of the differences in milk composition of the three common British breeds. The differences in butterfat per cent between Ayrshires and Friesians is almost entirely accountable to differences in milk serum yield. Comparing Ayrshire and Shorthorn, the lower butterfat per cent of the latter is due to both decreased fat and serum yields, the fat secretion drop being relatively greater.

The formula is advocated in further development of the milking point system in use in British Dairy Shows. Cox feels that the adoption of this method of analysis based upon the actual secretions of different constituents rather than utilization of derived figures such as butterfat per cent would do much to standardize methods of approach to milk secretion problems on a universal basis. J. D. Donker

480. The effect of prepartum milking on the composition of the prepartum and postpartum secretions of the cow. S. J. Rowlands, J. H. B. Roy, H. J. Sears, and S. Y. Thomson, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **20**, 1: 16-28. Feb., 1953.

Analyses by conventional methods were carried out on total solids, fat, solids-not-fat, lactose, total protein, ash, Ca, P, Cl, casein, albumin, globulin, proteose peptone, NPN, vitamin A, and carotenoids of milks from 8 Shorthorn cows which were prepartum milked. The analyses started with prepartum milking at an estimated 2 wk. before calving and continued to 4 d. postpartum. These samples were compared to the total colostrum obtained from 5 Shorthorns 1 d. postpartum.

It was found that the first obtained prepartum secretion was even higher in concentration of the constituents normally high in colostrum and lower than those normally low in colostrum. The trend from this extreme for this early prepartum secretion was toward normal milk. All constituents increased in total amount daily throughout the period, with the exception of the globulin. Globulin increased to a maximum at some variable time before or after calving and then rapidly decreased. This maximum point was closely associated with a highly increased rate of milk secretion. The prepartum milked cows and particularly the "responders" (volume of prepartum secretion of > 30 l.) had postpartum milk much lower in solids-not-fat, protein, ash, P, Cl, casein, globulin, albumin, and proteose peptones, but lactose was higher than in normal colostrum. Fat, Ca, and NPN in the secretions were alike.

The range of responses among the 8 cows on the volume of prepartum was from 1 to 96 l.; 5 being above 30. The mean day for maximum globulin production was 5 ± 1.5 d. before parturition. The daily vitamin A output was positively correlated to daily globulin secretion and negatively to that of daily fat output.

J. D. Donker

481. The milk-ejection response of the sow to standard doses of oxytocic hormone. W. G. Whittlestone, Ruakura Animal Research Sta., Dept. of Agriculture, Hamilton, N. Z. J. Dairy Research, **20**, 1: 13-15. Feb., 1953.

Six Berkshire sows were used in experiments in which a standardized intravenous injection of oxytocin (0.5 I.U.) was made periodically throughout their lactations. The results of 33 trials are reported. The oxytocin was administered into an ear vein and the time noted for the let-down response to be initiated. The duration of response also was noted. The average delay time was 15 sec.; the duration time, 27.5 sec. There were no trends in these times as the lactation advanced. The times for all sows were similar. J. D. Donker

482. Indirect determination of water retention in cattle as a means of estimating energy exchange. K. L. Baxter and J. A. F. Rook. Nature, **171**, 4353: 609. 1953.

Analyses of various organs, fluids, and fat depots of cattle, sheep, and rabbits, ranging in moisture content from 99% to <10% showed a definite relationship between the water content and the Na + K content. This relationship, found in foetal, young, and mature animals, was: water (g.) = 0.2922 Na (mg.) + 0.1471K (mg.). Only the contents of the abomasum failed to fit this equation, probably because of the low pH. Since the equation applied to individual tissues, the authors reasoned that it must apply also to the whole animal and to increments in the growth of the animal. The validity of this reasoning was tested by measuring the water retention of growing calves over an 8-day period by application of the equation and by carefully recording the total intake of water and subtracting the loss of water in the excretion. Data on 3 calves showed a moisture retention of 9,948 g. by the first method and 9,817 g. by the second. During this period, the total intake of water plus metabolic water was 113 kg. The authors suggest that the discrepancy is due to the aperiodicity of the excretions and that the difference would be less if the period of testing were longer. R. Whitaker

483. Combination of vitamin A and iodoproteins in relation to milk production. Bruno Mainardi, Univ. Mailand, Ger. Arch. Tierernähr., **2**: 265-9. 1952. (Chem. Abstr., **47**: 3939d.) S. Patton

484. Results of physiological and zoological investigations of the method of Usuelli-Piani for the use of nonfat milk for the nutrition of calves. G. Curto, Univ. Mailand, Ger. Arch. Tierernähr., **2**: 270-9. 1952. (Chem. Abstr., **47**: 3939g.) S. Patton

485. Influence of feeding milk and curd on the intestinal synthesis of vitamin B_1 in rats. S. Balakrishman and R. Rajagopalan. Indian J. Physiol. and Allied Sci., **6**: 143-54. 1952. (Chem. Abstr., **47**: 3943d.) S. Patton

486. Variations of the chief constituents of colostrum produced by cows treated with high doses of calciferol (vitamin D_2). R. Mantillaro, A. Bosticco, and A. Ubertalle. Atti soc. ital. sci. vet., **4**: 453-6. 1950. (Chem. Abstr., **47**: 3944d.) S. Patton

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

487. Bactericidal studies of chlorine. R. S. Ingols, H. A. Wyckoff, T. W. Kethley, H. W. Hodgden, E. L. Fincher, J. C. Hildebrand, and J. E. Mandel. Eng. Expt. Sta., Georgia Inst. of Technol., Atlanta. Ind. Eng. Chem., **45**, 5: 996-1000. 1953.

Chlorine has been widely used in water supplies for its bactericidal action, but the mechanism of this action is not well understood. Hypochlorous acid and chlorine dioxide appear to be effective because each forms an irreversible product from its reaction with the sulfhydryl radical. Since monochloramine does not do this, higher residuals and longer contact periods are required for complete bacterial kill. The condition or well-being of the organism at the time it is subjected to chlorine contact is shown to be important. B. H. Webb

488. Residual chlorine methods and disinfection of sewage. R. V. Day, D. H. Horchler, and H. C. Marks. Wallace & Tiernan Co., Inc., Newark, N. J. Ind. Eng. Chem., **45**, 5: 1001-1004. 1953.

The selection of a residual chlorine method that parallels disinfection is an important problem in sewage chlorination. A large variety and high concentration of organic and inorganic compounds in sewage react with chlorine, and this makes it difficult to use residual chlorine tests to control the bacterial quality of the effluent. This study of a large number of samples and their statistical treatment permits the selection of the proper chlorine method for a given purpose. B. H. Webb

489. Disinfection of wastes by chlorination. H. Heukelekian, R. Day, and R. Manganelli. N. J. Agr. Expt. Sta., New Brunswick. Ind. Eng. Chem., **45**, 5: 1004-1008. 1953.

This study was made to obtain the best chemical yardstick of residual chlorine. Residual chlorine indicated the effectiveness of chlorination for disinfection of mixtures of wastes and sewage in terms of the most probable number of coliform organisms that remained. Approximately similar magnitudes of residuals were obtained by the modified starch iodide and amperometric methods. Lower values were found for the o-tolidine method. B. H. Webb

490. The greater hazard—insects or insecticides. E. F. Knipling. Presidential address, 64th ann. meeting, American Assoc. Econ. Entomol., Dec. 15, 1952, Philadelphia. J. Econ. Entomol., **46**, 1: 1-7. Feb., 1953.

Alarmists apparently misinterpret laboratory toxicity data in terms of insecticide hazards to man. Toxicity hazard is largely dependent on how the insecticide is used. Entomologists have demanded toxicity data on insecticides before recommending them.

Calculated risks of using insecticides must be weighed against potential losses caused by insects, including death of millions of people each year, due directly or indirectly to insects. It is estimated that no less than 5,000,000 lives have been saved, and 100,000,000 illnesses pre-

vented, by using DDT in controlling insects which transmit organisms causing some diseases in man. Similar, but lesser, benefits are attributed to chlordane. There are 14 deaths recorded as due to DDT, but in most cases the solvent was a contributing if not the deciding factor. Chlordane has caused one death, due to occupational exposure.

Newer insecticides have nearly replaced more toxic arsenicals, fluorides, and some others formerly commonly used in homes. Insect resistance to insecticides may become more acute. The continuous necessity to recommend new insecticides demands increased practical toxicity hazard studies. The medical profession, with its recent wonder drugs as well as older ones, admitted that unexpected reactions may occur in spite of exhaustive research before release of the drugs. Entomologists, chemists, and toxicologists have cooperated in bringing us relatively safe insecticides, thereby improving health and living standards. E. H. Fisher

491. Chlorinated hydrocarbon insecticides to control house fly larvae. J. HADJINICOLAOU, School of Hygiene, Athens, Greece, and E. J. HANSENS, N. J. Agr. Expt. Sta., New Brunswick. J. Econ. Entomol., **46**, 1: 34-37. Feb., 1953.

Laboratory tests with house fly larvae showed that amounts as low as 2 mg. of aldrin, dieldrin, and heptachlor controlled them well when 25 ml. of emulsion was sprinkled on the surface of 500 g. of media. Chlordane, toxaphene, and dilan were slightly less effective. Methoxychlor, lindane, and DDT were much less effective.

Field tests against house flies in dairy barns were conducted by treating floors, gutters, calf stalls, and possible breeding sites outside the barn with either aldrin, heptachlor, or chlordane. This larval control alone was not sufficient for good fly control. Similar field tests were conducted on turkey manure and on a dairy manure pit. Good control was achieved at first, but effectiveness decreased with each application. Laboratory studies of field strains of adult and larval flies showed them resistant to all insecticides tested. E. H. Fisher

492. Development of resistance to insecticides in natural populations of house flies. I. H. GILBERT, M. D. COUCH, and W. C. MCDUFFIE, Bur. of Entomol. and Plant Quarantine, U.S.D.A. J. Econ. Entomol., **46**, 1: 48-50. Feb., 1953.

Female house flies, *Musca domestica* L., from dairy barns were laboratory-tested for resistance to some insecticides. Flies were exposed under petri dishes on plywood panels treated with 25 mg. of insecticides per sq. ft. Exposure time was that needed to cause 50% mortality.

One strain of flies, first showing resistance to DDT in 1948, were found in May of 1950 to be 12 times as resistant to DDT as laboratory flies, and 40 times as resistant by midsummer; they were more than 12 times as resistant to lindane, dieldrin, and aldrin, but not resistant to methoxychlor, parathion, or pyrethrum.

Increasing resistance of dairy barn flies from spring to fall was shown during 1950 and '51. Data are presented involving several insecticides, some also in combination with synergists. E. H. Fisher

493. Effect of temperature and the activity of house flies on their absorption of DDT. A. R. ROTH and A. W. LINDQUIST, Bur. of Entomol. and Plant Quarantine, U.S.D.A. J. Econ. Entomol., **46**, 1: 127-130. Feb., 1953.

Resistant house flies *(Musca domestica* L.) were treated on the thorax with 8 γ of radioactive DDT in acetone solution. They then were caged at constant temperatures of either 70 or 90° F. for 20 hr.

Average mortality in tests was 74% at 70° F. and 41% at 90° F. The average amount of DDT absorbed per fly was 64% greater at 90° F. than at 70° F. E. H. Fisher

494. Natural gas as a blowfly attractant. R. E. SIVERLY, Federal Security Agency, Public Health Service, Phoenix, Arizona. J. Econ. Entomol., **46**, 1: 156. Feb., 1953.

Blowflies of 3 species *(Phoenicia sericata, Phormia regina,* and *Eucalliphora lilaea)* were attracted to natural gas leaks. Females predominated, and eggs were deposited in debris near the gas leak. Probably hydrogen sulfide and butyl mercaptan, 2 trace constituents of the natural gas, served as ovipositional attractants. E. H. Fisher

495. Chlorinated polyphenyls to improve lindane residues. W. N. SULLIVAN and I. HORNSTEIN, Bur. of Entomol. and Plant Quarantine, U.S.D.A. J. Econ. Entomol., **46**, 1: 158-159. Feb., 1953.

Carbon tetrachloride solutions of lindane and a chlorinated polyphenyl were prepared separately and together in several concentrations. Solutions were poured on 1 sq. ft. panels of aluminum or masonite. The lindane was soon a white deposit, the mixture a clear residue which was tenacious and a little tacky, and the polyphenyl was hard and transparent. After 2, 10, 30, 60, and 90 d. of summer room temperatures, roaches *(Periplaneta americano* L.) were confined for 2 hr. under petri dishes on the panels.

Lindane residues of 50, 100, and 200 mg. per sq. ft. were effective (50% or better kill) for less than 15, 30, and 60 d., respectively. Effectiveness was much greater in the lindane-chlorinated polyphenyl mixtures. The polyphenyl alone caused no kill. The insecticides were much less effective on the porous masonite surface than on the aluminum. E. H. Fisher

496. Control of mosquitoes with DDT, aldrin, dieldrin, endrin, and isodrin. A. V. MITCHENER, The Univ. of Manitoba, Winnipeg. J. Econ. Entomol., **46**, 1: 164. Feb., 1953.

DDT controls mosquito larvae, but not pupae, when sprayed on breeding sites. Some adults thus emerge to become pestiferous.

Larvae and pupae *Aedes dorsalis* (Mg.) and possibly other *Aedes* spp. were subjected to laboratory treatments of 1 p.p.m. of either aldrin, DDT, dieldrin, endrin, or isodrin in the water. DDT was least toxic to both larvae and pupae. Endrin was especially effective against pupae. E. H. Fisher

497. Fly control on dairy farms. M. D. FARRAR and C. C. BRANNON, S. C. Agr. Expt. Sta. J. Econ. Entomol., **46**, 1: 172. Feb., 1953.

A bait containing 1.5 to 2.5 cc. of 40% tetraethyl pyrophosphate (TEPP), 4 fluid oz. molasses, and 1 gal. water was effective against all species of flies in dairy barns. It was applied lightly to clean barn floors by means of a sprinkling can, and was effective for 4 to 5 hr. Precautions in use of this hazardous material are given. E. H. Fisher

498. Milk sanitation honor roll for 1951-52. Anonymous. Pub. Health Reports, **68**, 4: 445-448. 1953.

A total of 239 cities and 56 counties are listed on the Public Health Service "honor-roll" of safe milk communities for the period from Jan. 1, 1951, to Dec. 31, 1952. A community must have a rating of at least 90% compliance with the USPHS code to be included on the honor roll. Separate lists are compiled for communities where milk sold is pasteurized and for those in which both raw and pasteurized milk are sold. D. D. Deane

499. Sterilization of milk utensils. O. BRATLIE. Tids. Norske Landbruk., 253-68. 1952. (Chem. Abstr., **47**: 4005f.) S. Patton

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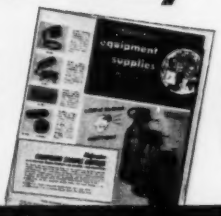

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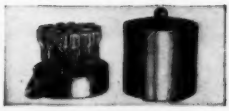

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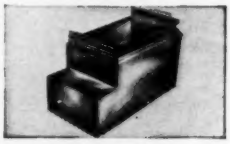

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